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CRITICAL COMMENTS ON MAMMALS FROM UTAH, WITH DESCRIPTIONS OF NEW FORMS FROM UTAH, NEVADA AND WASHINGTON

BY

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With the object of acquiring topotypes of mammals described from certain states adjoining Nevada, in order to make possible a better understanding of the systematic status of mammals now being gathered from that state by the Museum of Vertebrate Zoology, Miss Annie M. Alexander, accompanied by Miss Louise Kellogg, in 1929 and 1930, visited various localities and in Utah alone obtained 495 mammals. Although the collecting of topotypes was the principal objective of these collectors, other specimens were saved at the localities they visited.

The resultant collection, representing 68 species and subspecies, has provided 23 topotype-series of forms previously described from Utah; brought to light three new subspecies in that state; permitted the recognition of new forms in adjacent areas; added 11 other forms to the recorded mammalian fauna of Utah; extended the known geographic ranges of these and several other species; demonstrated intergradation between forms previously regarded as full species; and added otherwise, as set forth below, to our knowledge of western mammals.

Mephitis mephitis estor Merriam, Striped Skunk.—A female from Bluff, 4400 ft., San Juan County, is referred to this form which has not before been recorded from Utah. In certain respects it seems intermediate between the forms heretofore designated as *M. estor* and *M. mesomelas varians*. Possibly this specimen is an unusually broad-striped individual of *M. m. varians*. However, the tail is nearly white and therefore more suggestive of *estor*. If the specimen is correctly regarded as an intergrade it links up the several forms treated as subspecies of *mesomelas* with *estor*. As shown by recently acquired material, *estor* intergrades with *Mephitis occidentalis holzneri*. Intergradation probably occurs between *Mephitis mephitis nigra* (*Chincha putida*,

Howell, N. Am. Fauna, no. 20, p. 25, 1901) and *Mephitis mesomelas avia*, too. If this be so. we are required to use the specific name *mephitis* since it is the oldest of those proposed.

Mephitis mephitis major (Howell), Striped Skunk.—One skull-only which seems referable to this form on the basis of its large size (condylobasal length, 79.6 mm.) was obtained at Ephraim, Sanpete County. This occurrence provides a southern extension of range.

Urocyon cinereoargenteus scottii Mearns, Gray Fox.- -Bluff, 4400 ft., San Juan County, two skulls-with-skins and one skull-only; Blanding, San Juan County, 2 skins-with-skulls, one skin-only, and one skull-only. These specimens have the typically long tail of *scottii* and indicate a continuous range southward from the Uinta National Forest.

Canis estor Merriam, Coyote.—Practical topotypes are four skins-with-skulls and one skull-only from Bluff, 4400 ft., San Juan County; and one skin-only from Blanding, 6000 ft., San Juan County. Two skulls-only from Provo may be closer to *Canis lestes* than to *C. estor.*

The difficulties frequently encountered in exactly locating type localities designated in an earlier generation justifies presenting the information Miss Alexander was at pains to gather concerning Noland Ranch, San Juan County, Utah, the type locality of *Canis estor* Merriam and of *Peromyscus boylii rowleyi* (Allen). On November 16, 1929, it was learned from Mr. Noland, 77 years old and resident at Towaoc, Colorado, that the combined trading post and ranch bearing his name was established in 1873 on the north side of the San Juan River one and one-half miles above the present "Four Corners." This place is just over the Colorado-Utah line (about 2 miles as represented on a sketch map prepared by Miss Alexander, p. 29 of her note book on file in Mus. Vert. Zool.). A large part of the ranch has been washed away by changes in the course of the San Juan River, which necessitated three separate moves of the ranch buildings that now stand deserted on the mesa above the rim of the river. In 1929 few people, even in the immediate vicinity, were able to associate the name Noland Ranch with a definite geographic locality.

Callospermophilus chrysodeirus trepidus Taylor, Golden-mantled Ground Squirrel.—Two specimens from Pine Canyon, 6600 ft., Raft River Mountains, Boxelder County, constitute the first record of this form for the state.

Citellus armatus (Kennicott), Uinta Spermophile.—Represented by two specimens, one from Park City, 6970 ft., Summit County; and one from Pine Canyon, 6600 ft., Raft River Mountains, Boxelder County. The latter specimen is interesting as showing a large extension of range westward from the Wasatch foothills.

Ammospermophilus leucurus cinnamomeus (Merriam), Antelope Ground Squirrel.—The characters of a single specimen from St.

George, 2850 ft., Washington County, and the coloration of other specimens from farther south in Utah and Nevada indicate that specimens previously recorded from St. George as *A. l. leucurus* probably should be recorded as *A. l. cinnamomeus*.

Eutamias amoenus amoenus (Allen), Charming Chipmunk.—Ten specimens from Pine Canyon, 6600 ft., Raft River Mountains, Boxelder County, constitute the first record of occurrence of this species in Utah.

Eutamias umbrinus (Allen), Uinta Chipmunk.—Five near topotypes from southwest slope of Bald Peak, 10,500 ft., Uinta Mountains, and one from the South Fork of the Ogden River, 18 miles east of Ogden, represent this species. Miss Kellogg made the following interesting observation on this species.

We walked to a lake about a mile southeast of camp [= southwest slope of Bald Peak, 10,500 ft., Summit County] which we had seen from the summit of Bald Peak. On our way through the firs we saw a large chipmunk carrying something in her mouth that proved to be a young one. The mother held it in her mouth just in front of its hind legs and the fore paws of the young one were around her neck. She flattened out on a rock, not relaxing her hold on the baby, perhaps to get her breath after her trip of a hundred feet or more over logs and through the brush. Then she took it about twenty-five feet up in a big fir where she left it, ran down the branches and off in the direction from which we had first seen her come. We waited a few minutes but she did not return.

Eutamias dorsalis utahensis Merriam, Gray-backed Chipmunk.—Three young specimens taken June 21 and 22 at Beaver, 6000 ft., Beaver County, are instructive in that they show transitional stages in change of pelage. They are changing from the juvenile pelage to the adult, seemingly summer, pelage. The bright cinnamon, juvenile pelage is retained on the sides.

Thomomys perpallidus aureiventris Hall, Valley Gopher.—In addition to the type and eight topotypes from Kelton, 4225 ft., Boxelder County, there is an adult male, no. 44865 from Nephi, 5095 ft., Juab County, which agrees precisely in color with topotypes of *T. p. aureiventris*. Furthermore, in eight of eleven differential skull characters, as between *albicaudatus* and *aureiventris*, the specimen from Nephi agrees with *aureiventris*. In two skull characters, namely, shape of interpterygoid space and point of greatest bulge in zygomatic arches, it agrees with *albicaudatus*. In the eleventh skull character, size and shape of the lacrimal processes, the specimen is intermediate between the two. The general size and length of the claws on the fore feet appear to accord better with *albicaudatus* than with *aureiventris*. In the original description of *T. p. aureiventris* (Hall, Univ. Calif. Publ. Zool., 32, p. 444 [July 8, 1930]) the type locality was given as Kelton. Study of the collectors' field notes shows that this should be amended to 3 miles north of Kelton at the Fehlman Ranch.

Thomomys perpallidus albicaudatus Hall, Valley Gopher.—Beside the type and eleven topotypes from Provo, 4510 ft., Utah County, there are nine specimens from Beaver, 6000 ft., Beaver County. The series from Beaver shows some approach to *T. p. centralis* whose range lies to the west and southwest. The approach to *centralis* is reflected in

the color of the back, extent of white on the tail, degree of inclination of the jugals and in the size of the paroccipital processes. In two skull characters, namely, the posteromedially convex maxillo-frontal suture and the denticulate anterior margins of the nasals, the animals from Beaver are like *centralis*. However, in seven other differential skull characters they are like *albicaudatus*, as is true also of general size, color of underparts, and length of claws. Four of the specimens are young. These are lighter colored than the adults.

Thomomys perpallidus centralis Hall, Valley Gopher.—Four specimens from St. George, Washington County, seemingly are to be referred to this recently described form rather than to *T. p. aureus*, the name previously used for specimens from St. George.

Thomomys fossor Allen, Colorado Gopher.—Britts Meadows, 8500 ft., Beaver Range, Beaver County, 3; Brian Head, Mammoth Summit, 10,350 ft., Iron County, 4; Joshua Flat, 8340 ft., Elk Ridge, San Juan County, 3. The specimens from Joshua Flat seem to differ from true *fossor* as represented by four specimens from Pogosa Springs, Colorado, and two from Silverton, Colorado, in smaller size, slightly lighter color beneath, shorter and broader skull, posteriorly truncate rather than posteriorly rounded nasals, and in having lateral wings present rather than absent on the pterygoids. More specimens might show these differences to be inconstant and in any event specimens from farther west in Utah (Beaver and Parowan mountains), although they have wide skulls, at the same time have long rostra. On this account, despite the peculiarities of the specimens from Elk Ridge, no seemingly logical range for a new race of this wide ranging mountain top form is indicated by the available material.

Thomomys quadratus uinta Merriam, Brown Gopher.—Nine near topotypes from southwest slope of Bald Peak, 10,500 ft., Uinta Mountains, Summit County, and four from South Fork of Ogden River, 18 miles east of Ogden, Weber County, provide representative material of this mountain form. Outstanding differential characters distinguishing *uinta* from *quadratus* and *fisheri* are: slightly darker color; truncate rather than deeply emarginate posterior border of nasals; nearly parallel rather than posteriorly diverging temporal ridges; longer incisors; occlusal face of maxillary tooth row on a line with tips of incisors and ventral margins of tympanic bullae rather than produced below this line. However, a series of ten specimens recently procured by Miss Alexander from Albion, Idaho, shows intergradation of *uinta* with *quadratus fisheri*. A few of the ten specimens show varying combinations of the differential characters of the two forms, but the majority of the specimens show an intermediate stage of structural development as regards the individual characters themselves. Accordingly we are required to treat *T. uinta* no longer as a full species but instead as a subspecies of the earlier described *T. quadratus*. Bailey (N. Am. Fauna no. 39, p. 116) suggested that this might prove to be the case and although he too had specimens from Albion they were only three in number and so young as not clearly to demonstrate the intermediate structural features of the population there.

Thomomys quadratus fisheri Merriam, Brown Gopher.—Seven specimens from Pine Canyon, 6600 ft., 17 miles northwest of Kelton,

Raft River Mountains, Boxelder County, mark the first record of occurrence for this form in Utah. Indeed, on geographic grounds, it was supposed that *T. q. uinta* was the form inhabiting this area. This record of occurrence marks the easternmost one for this subspecies. Other mammals, collected in the Raft River Mountains, whose ranges like that of *T. q. fisheri*, are mostly to the southwest or west are: *Callospermophilus c. trepidus, Eutamias m. pictus; Perognathus p. olivaceus, Zapus princeps* n. subsp. (closely related to *nevadensis*), and *Neotoma. c. cinerea.* A more northern faunal complexion is provided by the presence of *Eutamias a. amoenus, Citellus armatus,* and the tendency of the *Neotoma c. cinerea* to dark coloration which is characteristic of *N. c. occidentalis.* The specimens of *Sorex p. navigator* and the presence of four other species of mammals noted by Miss Alexander, but not represented by specimens, namely, Porcupine, Mule Deer, Marmot and White-tailed Jack Rabbit, do not add much that is faunally diagnostic to the picture. The faunal position of the Raft River Mountains, as judged by Miss Alexander's and Miss Kellogg's mammal specimens, is clearly with the more southwestern Great Basin fauna of Nevada.

Dipodomys ordii columbianus (Merriam), Ord Kangaroo Rat.— Kelton, 4225 ft., Boxelder County, 5; Ogden, Weber County, 7; Provo, 4510 ft., Utah County, 10. With abundant material of *Dipodomys ordii columbianus* for comparison, I can detect no difference worthy of nomenclatural recognition as between *D. o. utahensis* (type locality, Ogden) and *D. o. columbianus.*

Dipodomys microps levipes (Merriam), Great Basin Kangaroo Rat.—Seven specimens from Kelton, 4225 ft., Boxelder County, mark the most northeastern record of occurrence for this form which has not before been recorded from Utah. *D. microps* of Owens Valley, California, and *D. levipes* of the Great Basin meet in the vicinity of Olancha and Darwin, Inyo County, California. Study of material from these two places indicates, to me, that actual intergradation does take place between the two. Accordingly, the two are treated as subspecies. This is the relation assigned to them by Merriam, the original describer.

Onychomys leucogaster brevicaudus Merriam, Short-tailed Grasshopper Mouse.—Five specimens from 6500 ft., 24 miles east of Salt Lake City, Wasatch foothills, mark a slight extension of range eastward in Utah.

Neotoma mexicana fallax Merriam, Mexican Wood Rat.—Eight specimens from Bluff, 4400 ft., San Juan County, provide a westward extension of range of this form which is not known to have been recorded previously from Utah. The extra triangle on M^1, the wide interpterygoid space, smaller bullae, and larger size distinguish this form from *N. i. desertorum.* So far as we know the Colorado River separates the ranges of the two. It would be worth while to collect along this river to see if intergrades occur anywhere. The facts that the two animals are externally so similar and that the ranges of the two species as now known are complementary would make this slender possibility worth looking into.

Neotoma cinerea cinerea (Ord), Bushy-tailed Wood Rat.—Two specimens from northwestern Utah, Pine Canyon, 6600 ft., Raft River Mountains, 17 miles northwest of Kelton, Boxelder County, are in process of molt. The new hair on the back is intermediate in color with that of *N. c. occidentalis* and *N. c. cinerea*, but the specimens seemingly are referable to the latter. Another specimen, from Taylor Canyon, Ogden, Weber County, is darker than specimens of *N. c. cinerea* from Nevada and California. Among the specimens available from Utah, the darkest of all are two in fresh pelage taken June 30 and July 1, at Britt's Meadows, 8500 ft., Beaver Range, Beaver County. *Neotoma cinerea* seems particularly responsive, in color, to climatic conditions. A positive correlation between dark color and high humidity probably exists and may explain the darker color of these Utah specimens as compared with the lighter colored populations from Nevada where more arid conditions obtain.

Neotoma cinerea orolestes Merriam, Bushy-tailed Woodrat.—A specimen taken by Mr. S. E. Aldous, at Moores Ranch, Ashley, 5 miles north of Vernal, Uinta County, establishes the range of this form as including northeastern Utah.

Phenacomys intermedius intermedius Merriam, Phenacomys.—This mammal, rare in collections and so far as known not previously reported from the state, is represented by four specimens taken from July 16 to July 25, 1929, on the southwest slope of Bald Peak, 10,500 ft., Uinta Mountains, Summit County. One taken July 25 is about half grown. It measures only 89 millimeters in total length. The collectors have noted that one of the specimens was taken among scrub fir, at the base of a large fir tree; another among the roots of a fallen tree sheltered by a thicket of fir; and a third was taken in the daytime among brush beneath a tree on a rocky hillside.

Clethrionomys gapperi galei (Merriam), Red-backed Mouse.—A young specimen from the southwest slope of Bald Peak, 10,500 ft., Uinta Mountains, Summit County, shows precise agreement in color with a slightly younger specimen, no. 2529, kindly lent for comparison by Mr. E. R. Warren, from Mud Springs, Garfield County, Colorado, which is regarded as *galei*. The two are near chestnut brown (of Ridgway, 1912). This specimen from Utah, which furnishes a considerable extension of range of *galei* to the westward, was trapped beneath young fir trees in a runway leading into a grassy spot with flowering plants all about.

Microtus nanus nanus (Merriam), Dwarf Vole.—Kelton, 4225 ft., Boxelder County, 2. Two other specimens of this interesting vole were taken at Logan, Cache County, by Professor J. S. Stanford, who placed them in the Museum's collection. These occurrences mark a considerable extension of range to the southwest.

Microtus richardsoni macropus (Merriam), Richardson Vole.—A single specimen taken on the southwest slope of Bald Peak, 10,500 ft., Uinta Mountains, Summit County, was trapped at the entrance to a hole, where runways with fresh cuttings were found, in a patch of skunk cabbage not more than ten feet square.

Ondatra zibethica osoyoosensis (Lord), Muskrat.—One skull-only from Bluff, San Juan County, marks a slight extension of range in Utah.

Zapus princeps cinereus, new subspecies

Type.—Female adult, skin and skull; no. 45422, Mus. Vert. Zool.; Pine Canyon, 6600 feet altitude, Raft River Mountains, 17 miles northwest of Kelton, Boxelder County, Utah; July 14, 1930; collected by Annie M. Alexander; original no. 689.

Diagnosis.—Coloration palest of the described forms of *Zapus;* sides pinkish buff (after Ridgway, 1912) mixed with black; size small; tail relatively as well as actually short; skull as in *nevadensis* except smaller.

Comparison.—As compared with a series of twelve topotypes of *nevadensis* donated to the Museum of Vertebrate Zoology by Mr. Ralph Ellis, Jr., *cinereus* differs in being decidedly smaller; tail relatively and actually shorter; entire coloration lighter; side of head between eye and nose whitish gray rather than pinkish buff mixed with black.

Remarks.—*Z. p. cinereus* is most closely related to *Z. p. nevadensis,* its nearest neighbor geographically, and is the palest, smallest, shortest tailed member of the *princeps* group. The two specimens first obtained were trapped by Miss Alexander and Miss Kellogg in sage brush, on a rocky slope, fifty feet above an aspen grove, where *Perognathus parvus olivaceus* also was trapped. The traps were then transferred to the small green undergrowth beneath birch and willow along the bank of the stream where the remainder of the specimens were taken. All but two of the nine specimens are young.

Specimens examined.—Nine from the type locality.

Examination of other material reveals two unnamed races of *Zapus* in Nevada.

Zapus princeps curtatus, new subspecies

Type.—Female adult, skin and skull; no. 7991, Mus. Vert. Zool.; head of Big Creek, 8000 feet altitude, Pine Forest Mountains, Humboldt County, Nevada; June 30, 1909; collected by W. P. Taylor and C. H. Richardson; original no. 777 of W.P.T.

Diagnosis.—Coloration pale; lateral line faintly indicated; posterior border of palate convex anteriorly; palatal bridge short; incisive foramina wide posteriorly.

Comparison.—As compared with adult topotypes of *Z. p. oregonus,* *curtatus* is slightly smaller in external measurements; has the sides of the head and back lighter; lateral stripe less well defined; zygomatic breadth less; much shorter palatal bridge; wider incisive foramina, especially posteriorly; narrower brain case; relatively larger rostrum, with nasals wider at the posterior end.

As compared with the other one of its two nearest relatives, *Z. p. major, curtatus* has the face lighter and is slightly smaller. Cranially it is distinguished by a more rounded skull, shortened in the poste-

rior part, with the brain case more inflated relatively to the anterior zygomatic structure; lesser zygomatic and mastoid breadth; shorter tooth row and palatal bridge; zygomatic arches more bowed out in posterior part.

Remarks.—Cranially, *curtatus* is nearest to *oregonus*. In color it is nearer *major*. Taylor (Univ. Calif. Publ. Zool., 7, p. 281, 1911) has given an extended account of the Pine Forest Mountain animals. He commented on their peculiarities, but without actual topotypes of *oregonus* for comparison did not feel justified in naming his specimens as distinct from the Blue Mountains form.

Specimens examined.—Total number, 18, from localities as follows: Head of Big Creek, 8000 ft., 13; Alder Creek, 6000 ft., 2; Meadow near Duffer Peak, 8400 ft., 1; and Leonard Creek, 6500 ft., 2, all in the Pine Forest Mountains, northern Humboldt County, Nevada.

Zapus princeps palatinus, new subspecies

Type.—Male adult, skin and skull; no. 45871, Mus. Vert. Zool.; Wisconsin Creek, 7800 feet altitude, Toyabe Mountains, Nye County, Nevada; May 26, 1930; collected by Jean M. Linsdale; original no. 3191.

Diagnosis.—Coloration pale; lateral line wanting; tail short; posterior border of palate straight or convex posteriorly; palatal bridge long; incisive foramina very wide posteriorly.

Comparison.—Indistinguishable from *nevadensis* in color, but tail and body shorter; posterior border of palate straight or convex posteriorly rather than convex anteriorly; incisive foramina averaging wider posteriorly.

As compared with *curtatus, palatinus* has grayer sides as a result of the lesser amount of yellow; palate straight or convex posteriorly rather than convex anteriorly; incisive foramina wider posteriorly with posterior border more nearly truncate; palatal bridge longer (no overlap in specimens examined); interorbital and mastoid breadths greater.

Remarks.—The nearest relative of *palatinus* is *nevadensis*. In two of the twelve specimens of *nevadensis* the palate is straight as in many individuals of *palatinus*. In one specimen of *palatinus* it is convex anteriorly as in *nevadensis*. Even in these three cases the two forms seem to be distinguished by the fact that the posterior border of the palate is on or near a line connecting the posterior margins of the last upper molars in *palatinus* whereas it is farther forward in *nevadensis*.

The generally straight, or even posteriorly convex, posterior border of the palate seems to be unique among the described forms of *Zapus*.

The name *palatinus* is given in allusion to this structural feature. The widening of the incisive foramina has been carried farther than in any other member of the genus. *Z. p. palatinus* marks the southernmost known extension of range of the species in the Great Basin.

Specimens examined.—Total number 14.   Kingston Ranger Station, 7500 feet, Lander County, 4; Wisconsin Creek, 7600 to 8200 feet, Nye County, 10; all from the Toyabe Mountains, Nevada.

Material of the genus *Zapus* accumulated by the Museum of Vertebrate Zoology from western North America now numbers 500 specimens.   Among these are a considerable number from British Columbia which show that *hudsonius* and *princeps*—employing the names in the specific or group, as opposed to the subspecific, sense—occupy common ground over a wide area in that province.   This overlapping of geographic ranges occurs without suggestion anywhere of intergradation between the two species.

Taking into account this fact together with the nature of the structural features separating the described forms, we seem justified in recognizing two groups within the subgenus *Zapus*, as opposed to the subgenus *Napaeozapus*.   These are the *hudsonius* group and the *princeps* group.   Possibly each is properly to be treated only as a species, with all the described forms arranged as geographic races of one or the other.

Be this as it may, within the *princeps* group there are three types of coloration: to the eastward in the Rocky Mountains the yellow-sided jumping mice, typified by *princeps;* to the westward in the Sierra Nevada, Cascades, and along the Pacific Coast, the salmon-sided forms, typified by *alleni, orarius,* and *trinotatus;* and in the intervening Great Basin, gray-sided, or perhaps more correctly stated, paler, pinkish-buff-sided forms.   These are *cinereus, nevadensis, palatinus, major, curtatus,* and *montanus.*   In these gray-sided forms the lateral line is faintly indicated or absent.   They are further characterized by having shorter tails than the yellow-sided mice in the Rocky Mountains.   The saturated coloration of the humid coastal forms is, of course, in line with color variation in other groups which similarly display a pale coloration in the arid Great Basin populations and a slightly less pale coloration in the less arid Rocky Mountains.

As arguing for the reduction in number of full species we may cite material from British Columbia which shows actual intergradation between *Z. saltator* and *Z. princeps.*   Other specimens from the Seven Devils Mountains, Idaho, recently donated to the Museum of Verte-

brate Zoology by Mr. Ralph Ellis, Jr, might be referred to *oregonus* or to *princeps* with almost equal propriety, although we place them with the latter. The three names, *oregonus*, *princeps*, and *saltator* certainly represent intergrading forms. Preble regards *minor* as another subspecies of this species. Although the Great Basin races are mainly isolated mountain forms which do not actually intergrade at present, they are nevertheless so closely related that it seems best to treat them all as subspecies of *princeps*. In morphological features *curtatus* in some respects bridges the gap between *oregonus* and *major* Z. *major* does not differ greatly from *palatinus*. Z. *palatinus* and *nevadensis* are similar, and *cinereus* resembles the latter. In each of these cases the differences are not greater than those which exist between subspecies in other groups where actual intergradation takes place.

On this account, or because of demonstrated intergradation, it seems best to regard, at least the forms listed below, with their type localities, as geographic races of the single species *Zapus princeps*.

Zapus princeps princeps Allen.   Florida, La Plata County, Colorado.

Zapus princeps minor Preble.   Wingard, near Carlton House, Saskatchewan.

Zapus princeps oregonus Preble.   Elgin, Blue Mountains, Union County, Oregon.

Zapus princeps saltator Allen.   Telegraph Creek, Stikine River, British Columbia.

Zapus princeps curtatus Hall.   Head of Big Creek, 8000 feet altitude, Pine Forest Mountains, Humboldt County, Nevada.

Zapus princeps major Preble.   Warner Mountains, Lake County, Oregon.

Zapus princeps palatinus Hall.   Wisconsin Creek, 7800 feet altitude, Toyabe Mountains, Nye County, Nevada.

Zapus princeps nevadensis Preble.   Ruby Mountains, Elko County, Nevada.

Zapus princeps cinereus Hall.   Pine Canyon, 6600 feet altitude, Raft River Mountains, Boxelder County, Utah.

Through gradual accumulation of material the Museum of Vertebrate Zoology now has suites of specimens, of *Microtus mordax* from California, Nevada, and adjacent regions, comparable as to seasonal state of pelage and age development of the skull. These permit the

MEASUREMENTS, IN MILLIMETERS, OF ADULTS OF SIX SUBSPECIES OF Zapus princeps. IN EVERY CASE THE SPECIMENS ARE FROM, OR VERY NEAR, THE TYPE LOCALITY. WHERE MORE THAN TWO SPECIMENS ARE AVAILABLE THE MEAN IS FOLLOWED BY MINIMUM AND MAXIMUM MEASUREMENTS IN PARENTHESES

	Total length	Length of tail	Length of hind foot	Occipito-nasal length	Zygomatic breadth	Interorbital breadth	Mastoid breadth	Height of skull	Length of upper molar-premolar tooth row	Length of palate
Zapus p major 10 ads	236 (221-255)	140 (132-150)	31.4 (28-35)	25.1 (24.3-26.4)	12.9 (12.0-13.3)	4.8 (4.6-5.0)	11.5 (11.2-11.9)	9.6 (9.4-10.0)	4.5 (4.3-4.8)	3.6 (3.2-3.8)
Zapus p oregonus 2 ads	230, 232	140, 135	32.0, 32.0	24.2, 24.5	13.0, 13.2	5.0, 50	11.1, 11.2	9.8, 9.4	4.3, 4.2	3.8, 3.8
Zapus p curtatus 7 ads	225 (213-235)	132 (125-141)	31.6 (30-33)	24.6 (24.0-25.3)	12.4 (12.2-12.7)	4.8 (4.6-5.0)	11.1 (10.8-11.3)	9.5 (9.1-9.7)	4.3 (3.9-4.4)	3.2 (2.8-3.4)
Zapus p palatinus 14 ads	229 (200-243)	134 (113-147)	32.6 (29-35)	24.6 (23.5-25.3)	12.6 (12.2-13.0)	5.0 (4.8-5.1)	11.4 (10.8-11.6)	9.6 (9.3-9.9)	3.6 (3.5-3.8)	3.6 (3.4-3.8)
Zapus p nevadensis 12 ads	244 (236-250)	143 (130-150)	32.7 (32-35)	25.1 (24.0-25.7)	12.7 (12.3-13.0)	4.9 (4.8-5.1)	11.4 (11.0-11.8)	9.6 (9.4-9.8)	3.7 (3.4-3.8)	3.6 (3.4-3.8)
Zapus p cinereus 2 ads	234, 235	135, 135	32, 32	24.1, 24.7	12.5, 12.5	4.7, 51	11.3, 11.1	9.2, 9.5	4.2, 4.5	3.6, 3.6

MEASUREMENTS, IN MILLIMETERS, OF ADULTS OF FOUR SUBSPECIES OF Microtus mordax. THE MEAN IS FOLLOWED BY MINIMUM AND MAXIMUM MEASUREMENTS IN PARENTHESES

Number of specimens and locality	Total length	Length of tail	Length of hind foot	Condylobasal length	Length of nasals	Zygomatic breadth	Interparietal breadth	Height of cranium and bullae	Mastoid breadth	Length of upper tooth rows
Microtus m sierrae 10 from Yosemite	196 (178-208)	69 (57-76)	22.2 (21-23)	28.2 (27.0-28.9)	8.5 (8.0-9.0)	15.8 (14.6-16.4)	8.4 (7.9-9.1)	10.0 (9.5-10.3)	12.7 (12.5-13.3)	6.7 (6.5-7.0)
Microtus m latus 10 from Toyabe Mountains	187 (180-197)	60 (52-70)	22.4 (21-24)	27.9 (27.2-29.0)	8.2 (7.8-8.9)	15.6 (15.0-16.0)	8.9 (9.2-9.6)	10.1 (9.8-10.4)	13.0 (12.6-13.4)	6.5 (6.0-6.8)
Microtus m mordax 4 from Seven Devils Mts	184 (179-197)	63 (60-68)	21 (20-22)	27.0 (26.1-28.3)	7.9 (7.0-8.0)	15.2 (14.8-15.7)	8.2 (7.8-8.4)	9.9 (9.7-10.1)	12.5 (12.2-13.1)	6.5 (6.1-6.9)
Microtus m angustus 2 from Blue Mountains	167, 178	48, 63	21.5, 19.5	27.4, 26.5	8.4, 7.5	15.4, 14.5	8.3, 7.8	9.8, 8.8	12.1, 12.1	6.5, 6.5

recognition of two new races, where previously, on account of less satisfactory material this geographic variation was not apparent, probably to Bailey (N. Am. Fauna no. 17, 1900), and doubtless to others who have studied the group.

Microtus mordax latus, new subspecies

Type.—Female adult, skin-and-skull; no. 45846, Mus. Vert. Zool.; Wisconsin Creek, 8500 feet altitude, Toyabe Mountains, Nye County, Nevada; May 28, 1930; collected by Chester C. Lamb; original no. 12409.

Diagnosis.—Size medium (see measurements); color pale; brain case very wide; rostrum depressed distally.

Comparison.—As compared with *M. m. mordax*, its nearest relative, *latus* differs as follows: body longer; coloration paler; skull longer; brain case broader (see comparative measurements of mastoid and interparietal breadths); nasals longer, averaging wider at tips, and noticeably more depressed toward the tips; incisors longer and heavier.

As compared with *M. m. sierrae*, *latus* is slightly smaller in external measurements; has the coloration markedly paler; brain case broader; interorbital depression absent rather than present; nasals shorter.

Remarks.—*Microtus mordax latus* exhibits the extreme of pallid coloration among the several subspecies except possibly *longicaudus*, specimens of which I have not examined. Kellogg (Univ. Calif. Publ. Zool., 21, p. 292) remarked on the pale coloration of specimens from the White Mountains of the California-Nevada boundary. This paleness of coloration reaches its extreme in central Nevada and is typified by specimens from the Toyabe Mountains. Specimens from other Nevada mountain ranges north and east of the Toyabes seemingly are referable to *latus* although they show tendencies toward typical *mordax*. Similarly the White Mountains population shows tendencies toward *M. m. sierrae* in both coloration and cranial characteristics. However, the White Mountains animals seem best referred to *latus*. Certainly this is true of specimens from low down on the desert floor of Fish Lake Valley at 4900 feet, which are paler than specimens from higher in the White Mountains and geographically nearer the Sierra.

Specimens examined.—Total number 55. White Mountains of Mono County, California, as follows: McCloud Camp, Cottonwood Creek, 9200 feet, 1; McAfee Meadow, 11,500 feet, 4; Big Prospector Meadow, 10,200 feet, 4; Poison Creek, 9000 feet, 2. White Mountains of Inyo County, California, Roberts Ranch, Wyman Creek, 8250

feet, 6. Esmeralda County, Nevada: Chiatovich Creek, 8200 feet, White Mountains, 4; Chiatovich Ranch [=Arlemont P. O.], 4900 feet, Fish Lake Valley, 3. Toyabe Mountains of Nye County, Nevada: South Twin River, 6500 and 7500 feet, 2; Ophir Creek, 6500 feet, 2; Wisconsin Creek, 7800 to 8500 feet, 17. Toyabe Mountains, Lander County, Nevada: Kingston Ranger Station, 7500 feet, 1; Birch Creek, 7000 feet, 4.

Microtus mordax angustus, new subspecies

Type.—Female adult, skin-and-skull; no. 40138, Mus. Vert. Zool.; Godman Spring, 5700 feet altitude, Blue Mountains, Columbia County, Washington; September 1, 1927; collected by S. H. Lyman; original no. H 23.

Diagnosis.—Size small (see measurements); color dark; brain case very narrow; highest point of skull near anterior end of frontals.

Comparison.—This form is nearly but not quite as dark as *M. m. angusticeps* and presents certain likenesses in the extreme narrowing of the skull. However, it differs from that form in that the bullae and incisors are as large as in *mordax*.

Remarks —The narrow brain case, heightening of the skull near the anterior end of the frontals, darker coloration, and seemingly small size distinguish *angustus* from *mordax*. This heightening of the skull near the anterior ends of the frontals causes the tips of the incisors and ventral faces of the tympanic bullae to lie in the same horizontal plane when the skull is placed top down. In available specimens of *mordax* the skull tips forward in this position and the tips of the incisors are lower than the ventral faces of the bullae.

The trenchant distinctions between *angustus* and *mordax* are the more remarkable because apparently typical *mordax* occurs in the Seven Devils Mountains, only sixty-five miles to the eastward. No doubt the gorge of the Snake River separating the two mountain masses is an effective barrier which prevents contact of the two populations.

The name *angustus* is given in allusion to the narrow brain case.

Specimens examined.—Six. Butte Creek, 4; Godman Spring, 1; Humpeg Falls, 1; all in Blue Mountains, Columbia County, Washington.

HYDROSTATICS OF THE SUCTORIAL MOUTH OF THE LAMPREY

BY

T. EMMETT REYNOLDS, S. J.

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CONTENTS

INTRODUCTION

During the course of some class work on the head muscles of the lamprey, my attention was directed to the unique structure of the tongue region of this primitive form. The tongue unit is made up of a number of complex elements that, by combining their functions, make that organ an efficient vacuum pump, or, when occasion requires, direct its anterior cutting edge against the body wall of the lamprey's host.

This suctorial adaptation plays an important rôle in the life-habits of the lamprey. In nesting, pairing, and food-getting, the lamprey is largely dependent on its ability to grasp and to hold by suction to external objects. The story of these life-habits is beyond the scope of this paper and has been adequately described (Gage, 1929, pp. 401–416). So far as I have been able to ascertain, however, the mechanics of the head and tongue involved in these various applications of hydrostatics has not received attention.

Acknowledgments

MATERIALS

About one hundred river lampreys were taken from the San Lorenzo River near the city of Santa Cruz, California, during the spring run. Nets were stretched diagonally across the swift little mountain stream and a fish trap was inserted in the 'rig' near the steeper bank. The ascending lampreys following along the net sought escape into this trap. The specimens were then refreshed with oxygen and hauled to the Steinhart Aquarium where they were preserved in fresh running water. It was not expected that they would live very long as they were near the close of their life-cycle, the spawning season.

Although a great number of dead and dying lampreys were examined during my work on the San Lorenzo River, scarcely any of the females had spawned or were even "ripe." They had met death prematurely from some unknown cause.

METHODS

Lipiodol Injections

I had hoped to observe the relations of the various movements of the tongue and suctorial disc by study of specimens under the x-ray. As a preliminary effort the hollow lobes of the tongue were injected with lipiodol, an oil apaque to x-rays, and the motion of these injected

areas was seen to follow the path of an arc, rather than a fore and aft motion. These movements were observed in the "screen" room and could not be reproduced photographically. The injections had a tendency to paralyze the tongue, and as a consequence the motions were of short duration. It is of interest to note here that the blood stream broke the oil injection into small globules and a succession of "deep" pictures taken at five-minute intervals showed the drops of oil following down the blood vessels, thus outlining them in dotted lines. The injection, of course, proved fatal to the specimen after about an hour.

BARIUM FEEDING

The progress of liquids between the tongue lobes and in the pharyngeal and branchial passages was next observed by feeding the specimen a heavy mixture of barium. The specimen was made to swallow barium in the following manner: A flask was fitted with a stop-cock and a pin hole was drilled along the side in a convenient position to accommodate the suctorial disc. It was found that the lamprey would strike at and affix the disc tenaciously to any smooth object brought near the head while the specimen was held in the hand. The flask filled with barium was presented to the lamprey, and after several efforts I was successful in slipping the disc over the minute hole. The stop-cock was opened to admit the air, and as a result the lamprey was forced to keep up a continued suction-motion in order to maintain its hold on the flask. A constant stream of barium through the pin hole was fed backward into the interior oral cavity, passing between the lobes of the tongue, flowing into the hydro-sinus and throat region, past the velum into the branchial tract, the gill pouches, and finally to the exterior through the gill apertures. None of the barium was observed in the oesophagus as this passage is sealed up early in the spawning season when the lamprey stops feeding. These observations made in the screen room could not be reproduced photographically, but two skiagrams made with high-power tubes are shown on plate 2, figures 2 and 5.

Gross dissections were made after the passages had been distended with petroleum jelly. After dissecting away the various muscles and head cartilages a hydro-sinus was revealed and photographed (pl. 1, fig. 4). It is believed that this structure is adequately figured and its function described for the first time in this paper. The same sinus flooded with barium is likewise revealed in the skiagram (pl. 2, fig. 5).

DESCRIPTION OF STRUCTURES

Tongue Elements

In plate 2, figures 1 and 3, a general idea is given of the tongue structure and the muscles actuating it. The tongue cartilage is shown in full length, the piston-like terminal, the paired muscles, the single heavy muscle with its long tendon. These muscles are employed in retracting the tongue terminal, rocking it about the flexible point of juncture with the long cartilage. I follow Tretjakoff (1926, pp. 267–304) in naming the muscles and cartilages.

It is important to observe that the long cartilage is practically fixed in position and takes up the thrust of the working member, the tongue terminal, or apicalis. This latter cartilage terminates in a slender thread of tendon buried within the cardio-apicalis muscle (pl. 3, fig. 2).

That the long tongue cartilage (glossa) is capable of little or no movement becomes evident from an examination of its structure and of its surrounding tendons and muscles. It can be moved forward slightly by the paired muscles, the basilari-glossus, that find their insertion on the glossal cartilage and their origin on the basilaris muscle (pl. 3, fig. 1). This glossal cartilage is easily traced between the paired basilaris muscles and it would seem that the reason of their peculiar structure is to steady this cartilage against side movement, or against buckling when back pressure is brought to bear upon it. The chief purpose, therefore, of the glossal cartilage is to furnish a firm base to support the cutting mechanism erected upon the apical cartilage and united to it by a hinge-like bond of tendon.

The trochlear structure of the apicalis furnishes a smooth surface upon which the several tendons slide back and forth in plying the cutting mechanism to be described. Three sets of tendons extend over this surface: the Y-shaped tendon of the cardio-apicalis and the two terminal tendons of the mandibularis apicalis muscles. All these tendons have their insertion on the ventral border of the apicalis.

The Apicalis

The apicalis (pl. 2, fig. 4) is shown vested with its cutting armament. The position of the right and left lobes of this member will suggest that they find their support on the right and left ramus of

the apicalis cartilage. The median border of this horny covering is fitted with a slight row of horny, saw-like teeth. The lower cutting edge is an independent member and moves independently of the lobes just described. It is equipped with a more serviceable row of teeth. It is this latter set of teeth that does most of the cutting when the cyclostome is gouging its way into the body wall of is prey.

The apicalis is divisible into a ventral and a dorsal segment. The ventral portion carries the main cutting edge. The dorsal segment is again divisible into right and left lobes. These lobes are separated by a cleft-like passage which permits the water and semi-liquid food to pass to the pharynx. In plate 2, figure 4, this rather complicated arrangement is illustrated. In this figure the tongue terminal has been dissected away from the oral cavity and photographed. The two lobes are supported on the cartilaginous framework (pl. 2, fig. 3). This framework is joined to the long shaft of cartilage by tendon and is readily moved about the flexible, joint-like bonding. It is well to observe here that the shaft is slightly offset toward the lower border of the apical cartilage and as a result any forward motion of the shaft results in an upward rocking movement of this member.

The tongue terminal is bound to the internal border of the mouth at this joint and is thus prevented from any appreciable extension outward or retraction as a unit within the oral cavity, but the dorsal tip of the tongue lobes can be rocked backward into the narrowing funnel of the mouth over a considerable arc. It is this rocking motion that cuts away the body tissue of the host and works the slimy mixture of shredded flesh and blood into the digestive tract; it is this same motion that partly controls the amount of water pressure within the annular lip and the consequent forces of attachment to outside objects by suction. The compressing of these tongue lobes closes the cleft-like passage and blocks the opening into the branchial and food passages. This compression is not effected by separate muscular action but by the mere drawing of the paired lobes into the rapidly narrowing throat passage.

In plate 2, figure 1, the position of the retractor muscles is shown. Their origin, as has been said, is on the lower border of the apical cartilage and they find their insertion by fascia and muscle, ultimately on the caudal terminus of the glossal cartilage. As a consequence, when these muscles are contracted a twofold motion results, the apicalis is rocked upward moving about the flexible union at the bond and simultaneously the long shaft is drawn a short distance forward.

The apicalis is returned to its position by the action of the paired muscles, the basilari-glossi muscles (pl. 3, fig. 1). These paired muscles, the innermost pair displayed by means of the wooden probe, find a common insertion on the T-shaped cartilage which ultimately attaches to the lower border of the tongue.

Hydro-Sinus

The fold marked hydro-sinus in text figure A, has been referred to by Rathke as the ''Gaumen-segel,'' or soft palate, but because of its function of storing water and ejecting it when necessary, I have termed it the hydro-sinus. This fold is shown in the reproduction of the x-ray skiagram in plate 2, figure 5, which represents a lateral view of the oral region after an injection of barium. Plate 1, figure 4, shows a photograph of the same sinus distended by petroleum jelly after the trabecularis had been dissected away. The sinus is surrounded by sturdy muscles and can eject forcibly a considerable volume of fluid.

Gage and others have observed that the lamprey ejects water violently through the branchial tract presumably to clear the passages of silt and foreign matter. I have noted that even after a great quantity of barium had been ejected through the gill apertures on either side of the body and the passages apparently cleared, the lamprey, after a lapse of ten or fifteen minutes on the x-ray table, would effect a secondary discharge of barium after a slight muscular spasm at which time the hydro-sinus would be emptied. From this it was concluded that the twofold function of this sinus was, first, to store a supply of liquid and eject it under pressure in order to clear the branchial tract, and secondly, to regulate the hydrostatic pressure in the oral region in conjunction with the action of the tongue.

One might object here that the hydro-sinus could scarcely hold a sufficient volume of water to clear the branchial tract effectively. It is quite true that the contents of the sinus would not suffice to fill the gill pouches and adequately cleanse them. The branchial tube, however, is a structure of small bore, and even a small quantity of water under sufficient pressure should prove effective in removing irritating particles of sand and foreign matter. One will recall that this tubular passage must accumulate an appreciable amount of sediment, since it is not exposed to the direct force of the stream that serves to aërate the gills. Furthermore, this passage is not equipped with constrictor

muscles and is incapable of automatically clearing itself of liquids and foreign matter. Finally, the rush of water through the oral passage when the lamprey is in motion is negligible, since the disc mouth collapses to a mere slit and takes a ventral position. It is therefore not exposed to the rush of water as the lamprey swims about. This constriction of the oral disc serves to stream-line the head and reduce the water resistance as the lamprey darts through the water. The lamprey takes first rank among the swiftest swimmers in our streams. From these considerations it is quite apparent that a structure such as the hydro-sinus for clearing out the inner branchial tract is admirably adapted to the need and is, in fact, the only structure to which this function may be assigned.
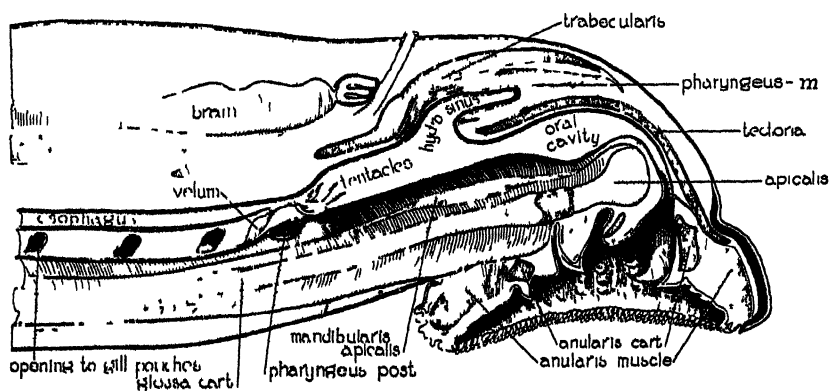
Fig. A. Semi-diagrammatic drawing of head region of lamprey showing relations of hydro-sinus, tentacles, and velum.

THE VELUM

The region between the velum (fig. A) and the oral disc must be maintained at a lower pressure than the pressure of the surrounding medium and also lower than the pressure in the branchial tract, if the lamprey is to feed successfully on its host, which is usually threshing about wildly during the early stages of the attack.

The reason is obvious; the branchial area is pumping water in and out of the gill pouches by the rhythmic contraction and expansion of the gill basket, hence this region represents outside water pressure. The passages anterior to the valve-like velum, however, shown semi-diagrammatically in figure A, are sealed off from the branchial region by the velum and represent a uniform, low-pressure area.

With this arrangement the tongue element is free to ply backward and forward in its twofold action of cutting and of pumping the blood and shredded flesh into the oral region. The solid matter is caught up by the tentacles and passed into the digestive tract while an excess of liquid taken in from the outside, which would imperil the suction-like hold, can be ejected past the velum by the contraction of the hydro-sinus, an optimum low pressure being restored by the consequent expansion of the sinus.

To sum up, the oral cavity is maintained at a minimum pressure by means of the valve-like arrangement of the velum and the coordinated pumping action of the hydro-sinus, which serves to exhaust this passage of leakage from the outside and thereby produces a low-pressure chamber in which the cutting mechanism is free to work much as a workman operating in a diving bell.

PUMPING CYCLE

The operation of feeding and the coordinated function of the suctorial disk, velum, and hydro-sinus of the lamprey in maintaining contact with its host are represented diagrammatically in figure B. Some of the details described in the pages to follow are necessarily inferential, as these operations transpire quickly and are of a nature very difficult to observe even with the aid of the x-ray.

The process of feeding barium through a minute aperture drilled in the wall of the barium flask, served not only to flood the oral and pharyngeal regions and thus render them visible under the x-ray, but also afforded a control on the motion of the elements involved and induced the specimen to repeat the act of suction until fatigue brought the motions to a stop. The lamprey held instinctively to the smooth walls of the glass flask and could do so without effort as long as no leakage occurred through the pin hole in the wall of the flask. But as soon as air was admitted into the flask through the stop-cock, the barium began to ooze through the tiny hole; this reduced the vacuum within the suctorial disk so that the lamprey was obliged to set up a sucking motion to maintain its hold. The specimen would collapse the annular lip against the glass and drive the liquid past the tongue lobes. The anularis muscle was then constricted and the disc warped away from the glass surface, thereby creating a new vacuum and in this manner securing attachment to the flask. By manipulating the

stop-cock control these muscular movements could be slowed down sufficiently to permit of leisurely observation.

The accretion of liquid barium was passed through the lobes of the tongue to the proximal oral region. When these areas were filled the stream apparently began to back up and filled the distal oral passages and slack folds of the hydro-sinus.

It would seem that the content of the oral cavity and pharynx, finding no other outlet, backs into the low-pressure region of the hydro-sinus. The constriction of this member increases the hydro-static pressure in the passage anterior to the velum to a point where it overbalances the rigidity of the velar valve and as a consequence a flow of liquid past the valve is effected. During this operation it would be imperative that the tongue block any passage of water anteriorly into the oral disc, as this leakage would imperil the attachment by suction of that member.

Further evidence that the tongue actually functions in this manner in conjunction with the constriction of the hydro-sinus was furnished by my observation of several lampreys clinging by the disc to the glass wall of a display tank in the Steinhart Aquarium. The specimen figured (pl. 1, fig. 3) was observed to flatten its disc against the glass, arching outwardly the upper segment while the tongue was drawn backward into the oral cavity. After patient waiting I observed that the lamprey began to slide the disc along the glass surface, at which time the tongue could be seen moving forward, and, at the end of this excursion, retracting to its old position, when the lamprey would come to a halt as if frozen to the window.

My interpretation of the forward motion of the tongue in conjunction with the slipping of the disc is as follows: in order to free the disc from the pressure of the surrounding water the inner pressure was increased to balance partially pressure on the external surface of the disc. This latter condition was effected by a flooding of the inner space with a stream of water admitted from the post-lingual region through the parted lobes of the tongue. The forward motion of the tongue permitted the lobes to part and the water was furnished from the post-lingual passage to which it was supplied under pressure by the constriction of the hydro-sinus.

In theory, the description just given, in which the flooded post-lingual region releases its contents into the partly evacuated oral disc, would apply not at the time of feeding, but rather in situations requiring instant contact and instant release of the suctorial disc. Such

situations are encountered by the lamprey in passing natural dams in swift streams—a process of attaching to rocks, releasing, darting forward, and attaching again. Furthermore in attacking its prey the lamprey must strike quickly, release, and strike again, thereby improving its position until the sought-for area is gained—the region rich in blood near the gills. It will be evident that these uses of the suctorial disc, which figure so prominently in the life of the lamprey, demand a high degree of flexibility in the manipulation of this suctorial disc.

On the other hand, during the interval of feeding, the post-lingual chamber is evacuated to the same degree as the disc and as a consequence there is no post-lingual supply of water available to flood the tightly attached disc. Release in this instance would be a slower process, the result of muscular contortion to raise some portion of the compressed border thus admitting a seepage of water from the outside. The only other possibility would be to elevate the velum and admit the water from the high pressure source in the branchial passage. From the structure of the velum and by reason of the head of water bearing against this valve, I am inclined to believe that this would be impossible.

The final phase of this feeding cycle finds, first, the hydro-sinus disgorged and ready for a new supply of liquid; secondly, the oral and pharyngeal region anterior to the velum at a lower pressure than at the beginning phase owing to the material discharged past the valve, the over-all volume of these several spaces having returned to normal after discharge with the relaxing of the pharyngeus anterior muscle.

In plate 1, figures 2 and 4, it will be seen that the hydro-sinus is encased in the dome-like cartilage, the trabecularis, and is free to expand into its arch without encountering back pressure, inasmuch as the trabecularis is a hard structure and under tank pressure does not collapse with the collapsing inner sack, the hydro-sinus. This condition is requisite if the system is to be cyclic, that is to say, continued increments of leakage passed through the tongue lobes must load the sinus and be expelled by its constriction, then reloaded and again expelled as often as this is necessary, in order to keep the chambers clear.

I have attempted a graphic summary of the pumping-feeding cycle in figure B, resolving these complex operations into four separate phases. The figures of the right column are diagrammatic, sagittal sections of a lamprey and those of the left column are intended as a mechanical illustration of the corresponding sections to the right. The

a

hydro sinus
velum
branchial passage
pharyngea region
cutting edge
collapsible-disc
tongue

Suctorial disc is collapsed against object, decreasing volume of its inner arch and driving water through the lobes of the tongue.

White area: low pressure
Broken lines: tank pressure

b

vacuum

Tongue closes passage between suctorial disc and pharyngeal region. The disc is warped outward increasing the volume and degree of suction.

c

vacuum

Hydro-sinus discharges its content through the velum while tongue continues to block oral passage.

d

residual water
vacuum

Velum blocks return of water from branchial passage. The discharge of hydro-sinus creates a low-pressure area from the velum to the oral disc. The tongue is now free to ply back and forth in cutting.

epiglottis
pharyngeal region
esophagus
velum
tentacles
gill pouch
branchial tract
hydro sinus
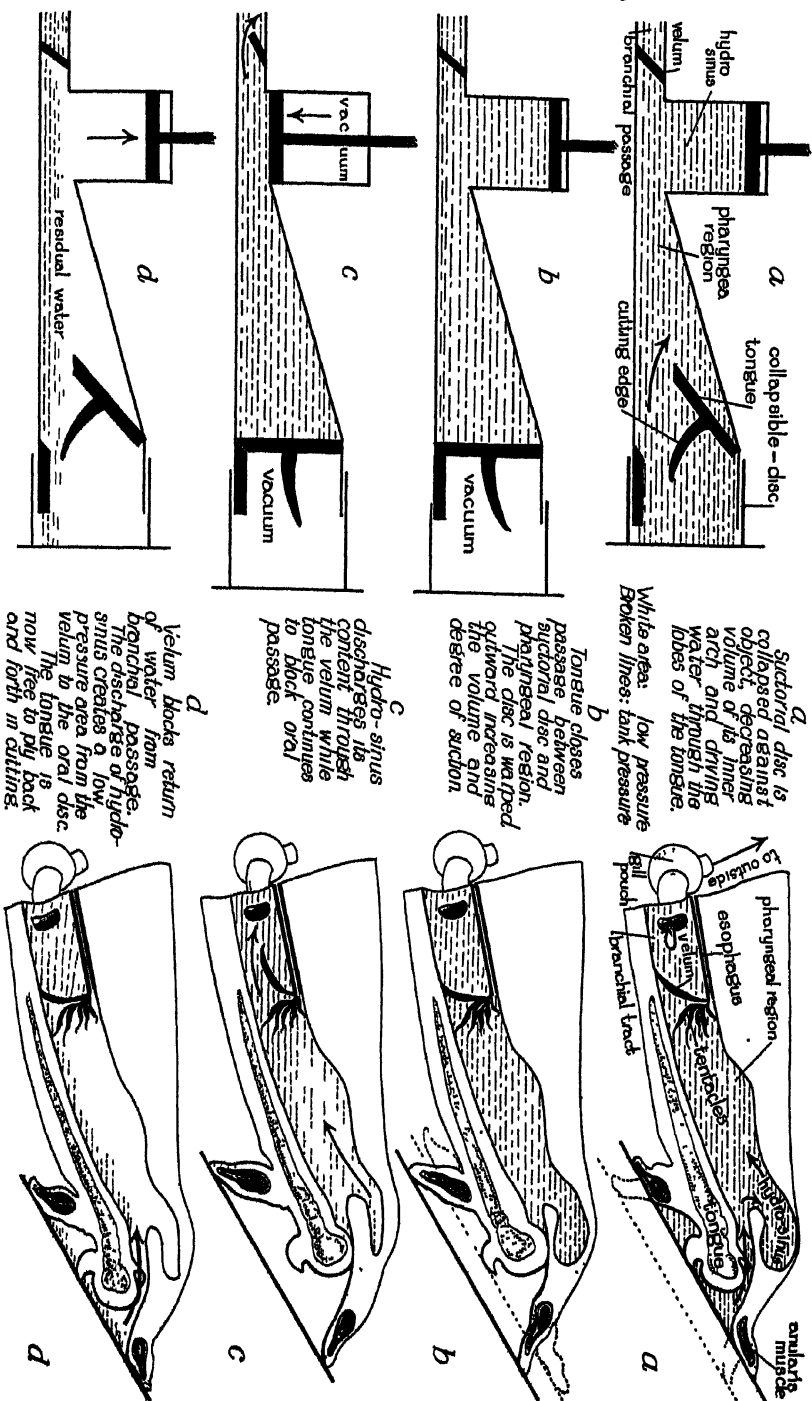tongue
annularis muscle

Fig. B. Diagrams of the four phases of the feeding-pumping cycle.

analogous parts are similarly labeled and the sequence of drawings in both columns represents successive phases in the action of feeding and of pumping water. The passages are drawn many times larger than natural size for the sake of convenience in labeling, and all details not dealing with our hydrostatic problem are suppressed.

The first phase (*a*) I have regarded as beginning with the compression of the collapsible disc against an outside object with a consequent discharge of its contents into the flooded, post-lingual region. The hydro-sinus is shown filled by this accretion since it is to be regarded as a region of minimum pressure, discharging and reloading continuously. For this reason the water expelled from the oral disc will find its way into the sinus rather than through the velum. This last statement gives rise to a difficulty which I think it well to dispose of at once.

It is true that the opening cycle (*a*) finds the passage in discussion at a uniform, tank pressure and one might conclude that water forced into the mouth would find its way past the velum, or into the rapidly filling, but not filled, sinus with equal ease; I do not think of another alternative. To this I answer that it is of no consequence which path the incoming water follows, provided only that the sinus is loaded and prepared for discharge in a subsequent phase. One must regard the loading and discharge of the sinus as a transient phenomenon, occurring in life continuously, perhaps, after the attachment of the disc-like mouth to its prey, hence at any instant save that of actual discharge a space of low pressure in the system.

The second phase (*b*) represents the action of the disc in enlarging its volume after the water has been expelled by warping away from the plate. This increase in volume is to be measured by the area between the dotted line and the heavy black line. The tongue is shown as blocking any back flow into this evacuated space. The situation thus far would find the lamprey attached to its host, but unable to employ the tongue in cutting its prey, partly because the disc in this position lifts the tongue away from the body wall, and partly because any motion forward of the tongue would result in flooding the rarified chamber formed by the disc. Quite obviously it will be necessary to reduce the pharyngeal pressure in order to free the tongue for its task.

This condition is met in the third phase (*c*) where we find the hydro-sinus discharging its contents past the velum, reducing the volume of water between the tongue and velum, thereby decreasing the pressure in the chamber. The velum is closed by the back pressure in

the branchial tract and a vacuum is produced in the hydro-sinus and connecting passages owing to the rigidity of the trabecular cartilage which prevents the collapse of the sinus.

In the fourth and last phase (*d*) the tongue is no longer required to block a flow of water from the pharyngeal region into the disc chamber as these two regions are now at a uniform, low pressure. The disc is now free to collapse somewhat and permit the tongue to engage in feeding. In life, it is quite certain that these phases have to be repeated almost continually owing to the fact that the oral disc is continually flooded with blood, slime, and seepage about the disc. Those who have seen a lamprey feeding will recall the continued working of the mouth and the quick darting of the head as it seeks to change or improve its hold.

A difficulty is encountered in the final phase (fig. B, *d*) in disposing of the mixture of liquids and solid matter in the pharyngeal region in such a manner that they will load the sinus. From the position of this structure in the diagram it appears that the sinus would not be loaded by gravity, rather the pharynx would serve as a catch basin for the accumulation of fluids and would in time fill and overflow into the disc. In this manner the suction hold of the disc would be diminished gradually.

From my observations in injecting these regions I am of the opinion that the pharynx offers higher resistance to the passage of fluids than the hydro-sinus, hence the fluids passing the cleft in the tongue will tend to load the hydro-sinus first and then be forcibly expelled through the pharynx by the contraction of the walls of the sinus as previously explained. My reason for this conclusion is drawn from the fact that in the process of injecting heavy oils through the cleft in the tongue I found that the hydro-sinus tended to fill before the pharyngeal region; because, this passage is collapsed normally and, although without constrictor muscles, is probably flattened out by the pressure of the surrounding muscles, swollen from the pressure of blood in the blood sinuses extending through these muscles.

SUMMARY

Studies made on living lampreys by means of the x-ray show a striking example of adaptation of body structures to fit the peculiar life-habits of these animals. By means of artificial feeding the movements of the various parts involved were controlled to permit of careful observation and their function determined during actual operation.

A hydro-sinus was discovered to be part of the hydrostatic equipment, serving as a storage chamber whence water could be forcibly ejected by contraction of the pharyngeus anterior muscle, in this manner cleansing the branchial tract of foreign substances. It is believed likewise that this hydro-sinus maintains the vacuum in the post-lingual region by ejecting leakage water and food past the velar valve.

A mechanical description of the feeding-pumping cycle is given. This description is based on the above observations as well as by inference from studies on the gross anatomy of the tongue and oral region.

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EXPLANATION OF PLATES

Fig. 1. Frontal section of lamprey showing the tongue dissected out and placed to one side revealing the entrance to the hydro-sinus (*Hs*). The tentacles and velum (*V*) are seen just beneath this entrance and mark the opening into the branchial tract. × 1½.

Fig. 2. Side view of lamprey with outer skin removed showing trabecular cartilage (*Tr*) before it was cut away. The engorged hydro-sinus (*Hs*) lies beneath the trabecularis. The underlying cartilage (*Te*) is the tectoria; the muscle (*A*) is the anularis. × 1½.

Fig. 3. Photograph of a living lamprey taken through the glass wall of the display tank as it hung suspended by its suctorial mouth. Note the lower lip flattened because of the pressure of the water. The tongue is shown in a retracted position. × 1.

Fig. 4. Front view of trabecularis (*Tr*) partly dissected away showing the hydro-sinus. × 1½.
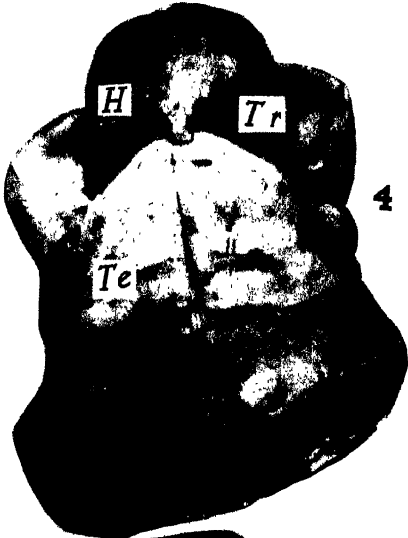
Fig. 5. Same view as in figure 4, before dissection.
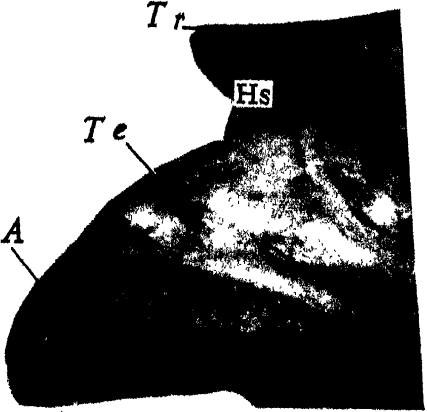
1

3

4

5

PLATE 2

Fig. 1.   The arrangement of muscles and cartilages of the tongue are shown much enlarged.   The mandibularis muscle is indicated by the wooden probe.

Fig. 2.   Dorsal skiagraph of lamprey.   Passages partly flooded with barium.

Fig. 3.   The tongue cartilage.   × 5.

Fig. 4.   The apicalis.   × 5.

Fig. 5.   Lateral skiagraph of the oral passage of lamprey after injecting the specimen with barium.   (*N*), partly filled passage to nasal sac; (*Hs*), hydro-sinus; (*Ve*), velum; (*Oo*), oral cavity.
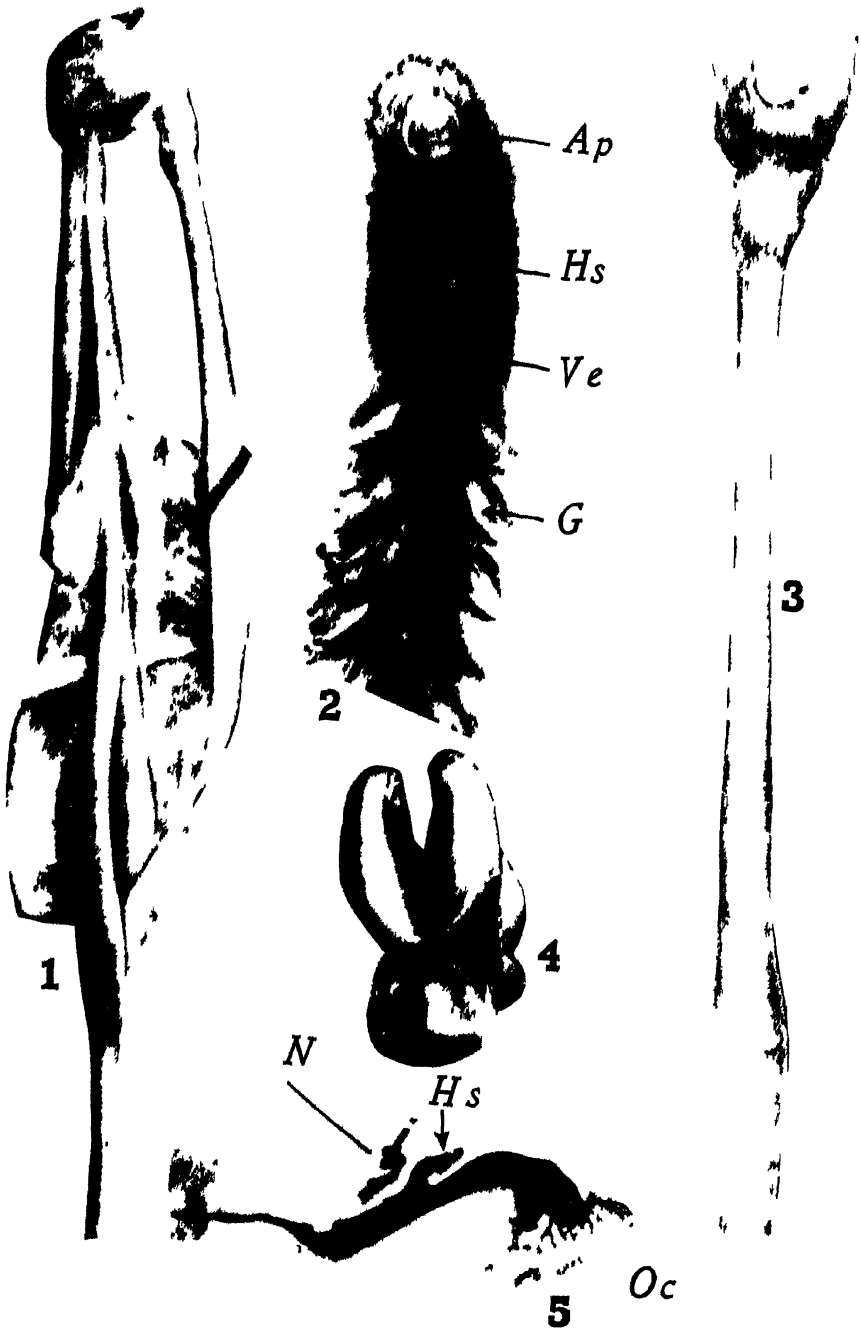
PLATE 3

Fig. 1. Ventral aspect of superficial musculature. × 2.

Fig. 2. Same view as in figure 1. Profound musculature. (*Gc*) glossal cartilage can be seen projecting from the paired basilaris muscles. × 2.

VEINS IN THE ROOF OF
THE BUCCOPHARYNGEAL CAVITY OF
SQUALUS SUCKLII

BY

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INTRODUCTION

Although there is a voluminous and complicated net of veins in the roof of the buccal cavity and pharynx of *Squalus sucklii*, these rarely appear upon the ordinary methods of injection. In some three thousand specimens injected forward through the posterior intestinal vein practically none had these vessels filled. In case a two-way injection was made and the second one backward into the orbital sinus, this system was occasionally filled.

MATERIALS AND METHOD

The specimens of *Squalus sucklii* herein studied and described were injected with a red starch mass through the caudal aorta in order to fill the arteries, and with a gelatin mass colored with chrome yellow in order to fill the veins. Four portals for venous injection were used: (1) the orbitonasal vein, (2) the dorsal cutaneous vessel, (3) the dorso-median rostral vein (see *dm.v.*, fig. 1), and (4) the subrostral vein (see *sr.v.*, fig. 2).

The orbitonasal vein was reached through the orbitonasal canal in the anterior angle of the orbit; it furnished a satisfactory way to fill these vessels. The dorsal cutaneous was found to be one of the best portals to use. A fine canula was inserted forward into that segment of the vessel which lies between the first dorsal fin and the endolymphatic ducts. From this injection the mass readily flowed forward and downward to the veins in the buccal and pharyngeal roof. The dorsomedian rostral vein, although somewhat smaller, likewise served as an advantageous portal for injection. This vessel lies in the mid-dorsal line above the rostrum and was located by removing the integument covering the anterior fontanelle. The subrostral veins

on the ventral side of the rostrum, in their posterior segment are of fairly good size and were readily injected both forward into the snout and backward into the buccopharyngeal area.

In order to expose the roof of the oral and pharyngeal cavities after the injections had hardened, the mackelian cartilages and the epibranchial segments of the visceral arches were cut through on both sides and removed. By further removal of the skin from the roof of the mouth we were enabled to study the position and pattern of the buccopharyngeal veins and their tributaries and relations.

THE VEINS

Figure 3 gives a general view of the veins in the roof of the buccal cavity and pharynx. These consist of a pair of large vessels (*bp. v.*) on the right and left sides of the roof, each of which runs along between, but ventral to, the hyoidean efferent artery (*hy. ef*, Daniel, 1928) and a paired dorsal aorta (*d. a.'*). In the posterior part of the pharynx these two vessels approach each other but do not meet. In the anterior part of the roof they meet and fuse above the symphysis of the upper jaw as a large median sinus (*s.*, figs. 2 and 3). By the removal of a median segment of the upper jaw on which the median dorsal teeth are situated the position and extent of this sinus may be made out.

For convenience of description we may follow the tributaries of this sinus forward and upward to their sources and then, in more detail, follow these vessels back down to the sinus (*s.*) and backward over the roof of the buccal and pharyngeal cavities to the position where they finally empty into the anterior cardinal system.

The sinus (*s.*) is found to be made by the confluence of right and left nasomaxillary veins (*nm. v.*, fig. 2) which, if we follow on one side we soon find joined by a subrostral vein (*sr. v.*) coming back from the ventral side of the rostrum. We may continue the nasomaxillary upward around the olfactory or nasal capsule (*n. c.*) and through a foramen in the cartilage to the dorsal side of the cranium. Here it becomes the supraorbital vein (*so. v.*, fig. 1) which is entered by one or two dorsolateral rostral veins (*dl. v.*) and is continued posteriorly over the supraorbital crest.

The supraorbital vein is a direct continuation of the dorsal cutaneous vein after its division as described by Daniel and Stoker (1927). In addition to the connection with the orbital sinus described

Fig. 1. Veins dorsal to roof of cranium, Squalus sucklii.

bp.¹, buccopharyngeal vein; br.¹, first pharyngobranchial cartilage; cn., entrance of buccopharyngeal vein into anterior cardinal system; d a.¹ and d.a., paired and unpaired dorsal aortae; dl. v., dorsolateral rostral vein; dm. v., dorsomedian rostral vein; fn., fenestra; fo. VII, exit of superficial ophthalmic nerve; hy. ef., hyoidean efferent artery; lls, superior levator labialis muscle; n.a., nasal aperture; n. c., nasal capsule; nm., nasomaxillary vein; n. t., nasal vein; on. c., orbitonasal (facial) vein; p. f., parietal fossa; po. p., postorbital process; s., sinus; so. t., supraorbital vein; sr. t., subrostral vein.

Fig. 2. Veins in roof of buccal cavity, Squalus sucklii.

Fig. 3. Veins in roof of buccal and pharyngeal cavities, Squalus sucklii.

by Daniel and Stoker, each supraorbital has one or two additional branches emptying into the orbital sinus.

If we now retrace our steps we shall find that most of the blood from this area passes down to and empties through the buccopharyngeal into the anterior cardinal system. The supraorbital vein (*so. v.,* fig. 1) on each side collects blood from the skin and the jelly-like tissue overlying the cranium, and each vessel then extends forward from the segment of the parietal fossa (*p. f.,* fig. 1) in a separate sulcus longitudinalis and then over the dorsal surface of the nasal capsule. At the place where the ophthalmicus nerve (*fo. vii.,* fig. 1) perforates the cartilage the left supraorbital receives the dorso-median rostral (*dm. v.*). On the right side at about the same position in the specimen figured it receives the median branch of the dorsolateral rostral (*dl. v.*) which comes from the dense gelatinous tissue along the anterior cartilaginous rostral bar. The other ramus of the dorsolateral vein runs along the dorsal margin of the rostrum. Both of these vessels may unite to enter the supraorbital vein as a common stem (*dl. v.*). If entrance be made by a common stem it is above the nasal capsule and near the foramen through which the supraorbital perforates the cartilage. If entry be by the two branches separately the outer branch enters near the foramen in the roof of the nasal capsule.

The supraorbital vein after passing through the foramen in the roof of the olfactory capsule continues as the nasomaxillary (*nm. v.*). As this vein curves downward within the capsule and along its posterolateral aspect it receives a fairly large branch (*n. v.,* fig. 2), which is the resultant of a remarkable leash of vessels coming from the folds of the olfactory organ. Just after the nasomaxillary enters the cartilage in the posteroventral region of the capsule it is joined by the orbitonasal vein (*on. v.,* O'Donoghue, 1914 and 1928; facial vein, Parker, 1886), which serves to connect the veins under discussion with the orbital sinus, a part of the anterior cardinal system. The nasomaxillary vein then emerges from the nasal cartilage just laterad of the point where the maxillary nerve first passes under the basal fenestra (*fn.,* fig. 2, Wells, 1917). Here it receives not only twigs from the skin and tissue outside of the capsule, but also longer branches from the tip of the snout, which we have designated the subrostral vessels (*sr. v.*). In the posterior part of its course the nasomaxillary vein swings caudad under the maxillary nerve, then ventromediad in front of the superior labialis muscle (*lls.*) to join its mate from the opposite side to form the dorsal symphysial sinus (*s.*) from which we started.

The buccopharyngeal veins (*bp. v.*, fig. 3) proper begin at the posterior end of the sinus (*s.*), and at the basal angle of the cranium right and left vessels swing outward following the margin of the orbits. At the postorbital process each buccopharyngeal receives one or two lateral tributaries form right and left sides of the upper jaw, and then right and left vessels take an almost parallel course posteriorly to the segment of the third branchial arch. Posterior to this the trunks converge anterior to the oesophagus, from the dorsal anterior wall of which they collect blood from a terminal plexus.

These buccopharyngeal trunks further receive blood medianly from a plexus of veins lying between them. Some of these minute branches run parallel with the paired dorsal aortae (*d. a.'*, fig. 3) and empty into the anterior segments of the buccopharyngeals where the latter diverge from the sinus. Their primary loci of entry, however, appears to be posteriorly in the segment of the main veins which extend from the first pharyngobranchial to the hyomandibular cartilage. In this particular region slightly larger transverse branches may sometimes be observed crossing from one side to the other.

Most of the veins associated with the spiracle, as for example, the large vein arising from a series of veins in the anterior wall, enter the anterior cardinal system direct; some of the smaller branches, however, in the anterior and in the posterior walls open into the longitudinal buccopharyngeal trunks laterally.

The buccopharyngeal trunks finally empty their blood into the anterior cardinal system by at least two connectives (*cn.*) on a side lying between the second and third and third and fourth pharyngobranchial cartilages (*br.*, fig. 3), respectively. Of these the large connective between the second and third branchial arches courses about one centimeter dorsad to enter the anterior cardinal sinus.

SUMMARY

Through the portals of the injection listed at the beginning of this article we have been able to demonstrate a remarkable group of veins in the roof of the buccal and pharyngeal cavities of *Squalus sucklii*. These vessels consist primarily of two large parallel trunks, which we have designated as the buccopharyngeal veins, and certain important tributaries, draining the dorsal part of the cranium in the region of the orbit, the rostrum both dorsally and ventrally, and the larger part of the olfactory area. This group of veins is connected with the cutaneous system of veins through the dorsal cutaneous, and with the orbital sinus of the anterior cardinal system through the orbitonasal vein. These vessels finally empty their blood into the anterior cardinal sinus in the roof of the pharynx.

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FEATURES IN THE DEVELOPEMENT OF AMMOCOETES

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INTRODUCTION

Ammocoetes, the larval form of the lamprey, furnishes an abundant source of chordate material along the Pacific coast. Lampreys travel up practically every stream which reaches the ocean, seeking nesting places. During and after the spawning season thousands of these animals may be found dead along the courses of these streams, and their larvae, Ammocoetes, may be taken in different stages of development from the sand or mud along the banks. These I have found during the months of May and June from the tributaries of the Eel River in the north to the San Lorenzo and Carmel rivers in the middle part of the California coast.

While availability is important in embryological material, it is, of course, by no means so important as the character of the material itself. As material Ammocoetes is superb. If one is interested in natural history, it would be difficult to find an animal in which the changes are more interesting than are those occurring at the time when the larva metamorphoses into the adult form. If one turns to the earlier phases of larval life, there is available a type which is so nearly unobstructed by pigment or by density of layers that it may be viewed under low power of the microscope as a semi-transparent object. In such stages one may study in the living animal both its structure and its activity. The structures involved in a function like respiration may be observed in detail, down to the individual muscle fibers of the velum; and their activity may be studied over long periods of time. The heart and the vessels associated in the circulation of blood may be observed clearly so that every phase of heart beat and of the course of the blood through such organs as the liver, and even the brain, may be followed.

THE WHOLE MOUNT

In a side view of a stained specimen from five to twelve millimeters in length the different regions, systems, and organs of Ammocoetes may be examined (fig. 1). Certain systems or organs extend through two or more regions. Such is the case with the myotomes (*my.*), or musculature, with the notochord (*ch.*), with the digestive tract (*vi.*), and with the central nervous system (*nt.*).

A. The Head

The oral hood (*hd.*, fig. 1) in Ammocoetes has a moderately well developed dorsal lip, but owing to the fact that it is not carried forward by the notochord, as it is in Amphioxus, the hood is relatively much blunter in the former than in a similar stage of the latter. In a younger larva of Ammocoetes, say of three millimeters in length, the hood has not yet formed, although it is represented by the area below the nasal pit; at that stage the nasal pit is ventral in position and marks the anterior extent of the head.

The hood has attached to its roof and sides numerous more or less branched oral papillae. Some of these occupy a position similar to that of the wheel organ of Amphioxus described by Johannes Müller. According to Müller, the wheel or Räderorgan possesses long cilia which drive a current of water back into the pharynx. In Ammocoetes, as we shall see later, the respiratory current is produced by the active beating of the velum (*vl.*, figs. 1 and 2).

The nasal pit (*np.*, fig. 1) in a larva of five millimeters or older occupies a dorsal position, separating the hood from the head proper. Seen from above in a living specimen, it takes a median position just anterior to the olfactory lobes (*ol.*), is roughly shaped like a figure 8 in surface view, and in deeper section has the apex pointing posteriorly. The pit extends downward and backward as a funnel almost to, but not breaking through the roof of the mouth. The cells lining the funnel are ciliated and often continue their activity after other signs of life have ceased.

The eye (*e.*, fig. 1) in Ammocoetes is formed early in the embryo, but it soon sinks under the skin, where it remains for three or four years, or until metamorphosis. At the end of this time the eye approaches the surface as a functional organ.

Fig. 1. Photograph, whole mount 9 mm. Ammocoetes.

a., anal aperture; *cb.*, cerebellum; *ch.*, notochord; *e.*, eye; *en.*, endostyle; *fd.*, fin fold; *gb.*, gall bladder; *hd.*, oral hood; *ht.*, heart; *l.*, liver; *my.*, myotome; *np.*, nasal pit; *nd.*, neural tube; *oe.*, oesophagus; *ol.*, olfactory lobe of brain; *ot.*, otic or ear vesicle; *pr.*, prosophagus; *pt.*, postvalvular segment of intestine; *vi.*, valvular intestine; *vl.*, velum.

The ear vesicle (*ot.*, fig. 1) is relatively large, but its definitive parts have not developed at this stage.

The buccal cavity (*bc.*, fig. 2) is entered through the mouth (*m.*) and is that part of the digestive tract anteriorly which is lined with ectoderm in the embryo and separated from the pharynx by the velum (*vl.*, figs. 1 and 2).

The velum is composed of right and left halves or bands which are attached ventrally to the floor just in front of the endostyle (*en.*, fig. 1), and laterally to the walls. These bands are arched upward and forward to attach also dorsally to the cranium between the ear vesicle and the eye. Each half of the velum at rest, if seen from the pharynx, as in figure 2, resembles a hand with the free margin like the fingers partly closed. It is provided with numerous parallel muscle fibers which run along its external margin, and other fibers which run obliquely to these. The stroke of this organ is made by the synchronous contraction of these muscles, bringing the inside free margin of the bands posteriorly and outward like a cup or pocket. These strokes are made with great vigor and maintained at a fair degree of regularity under a given condition. By the activity of the velum and the pharyngeal walls the respiratory current is drawn into the buccal cavity, down the pharynx into the branchial pouches, and forced out through the external branchial apertures.

B. The Pharynx

The pharyngeal area extends from the velum (*vl.*, figs. 1 and 2) to the oesophagus (*oe.*, fig. 1) and contains the ciliated peripharyngeal grooves (*pg.*, fig. 2), the gill pouches (*gp.*), and the endostyle (*en.*).

The peripharyngeal grooves were discovered by Anton Schneider (1879) and appear to represent a pair of gill pouches anterior to the present first pair of pouches. This pair of pouches in the embryo fails to break through and its walls become ciliated. Right and left peripharyngeal grooves (*pg.*, fig. 2) begin at the anterior margin of the endostyle and are connected posteriorly with the slit-like aperture (*en.*, fig. 2) of the endostyle. From the endostyle the grooves (*pg.*) pass forward and swing outward and upward in the wall of the pharynx along the first pair of visceral arches; they then approach the mid-dorsal line on the roof of the pharynx. Finally grooves in a mid-dorsal ridge extend posteriorly to the oesophagus.
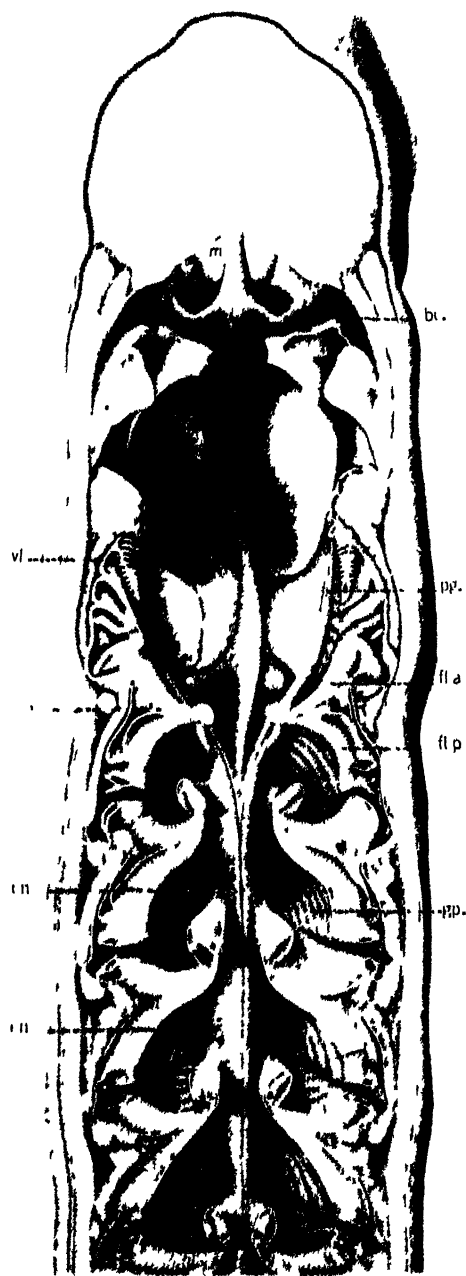
Fig. 2. Head and upper part of pharynx with roof removed, older larva, Ammocoetes, dorsal view.

bc., buccal cavity; *cn.,* aperture from endostyle; *en.,* endostyle; *fl.a., fl.p.,* anterior and posterior filaments respectively of holobranch; *gp.,* gill pouch; *m.,* mouth; *pg.,* peripharyngeal grooves: *s.,* septum: *vl* velum

In Ammocoetes there are seven gill pouches (*gp.*) on a side, only five of which are shown in figure 2. These may be regarded as a longitudinal series of rooms or cubicals, separated from one another by septa (*s.*, fig. 2). These septa thus originally form the anterior and posterior walls of the individual pouches.

Figure 2 of an older larva (2.5 *cm.*) shows the basket-like lining of the pouch. If we take the third pouch (*gp.*) for a detailed study of this lining, we observe that it is produced by anterior and posterior gill filaments which run from their septa as a series of parallel folds and decrease in length the more ventrally, or dorsally, their position. Externally anterior and posterior filaments approach one another, but they remain separated by the space through which the water leaves the pouch.

If, instead of considering the gill pouch, we consider the holobranch as a unit (Daniel, 1928), each holobranch consists of an anterior demibranch, comprising the filaments attached to the anterior face of the septum (*fl.a.*, fig. 2) and a posterior demibranch including the filaments (*fl.p.*) joining the posterior surface of the septum.

Individually, the filaments (fig. 2) on the first septum are prominent folds which extend posteriorly into the pouch. On the remaining gill septa, excepting the last, which is not shown in figure 2, filaments are present, as we have seen, both on the anterior (*fl.a.*) and on the posterior (*fl.p.*) faces of the septa. Those on the posterior face are better developed than are those on the anterior, and extend farther posteriorly and are less oblique to the axis of the body.

A transverse section through the pharynx gives a complicated picture of the gill structures (fig. 4), which can be better interpreted if studied in relation to figure 2. In this section twelve gill filaments (*fl.p.*) are indicated on the left side. On the right the section cuts through the whole width of the attachment of the filaments (*fl.a.*) of the anterior demibranch and then through the filaments in the posterior demibranch before reaching the pharynx. Had the section been cut farther backward on this side, it would have shown only the septum and the filaments of the posterior demibranch, as is indicated at the top of the pharynx on the left side. Each filament in this more mature larva is seen to be folded on its dorsal and ventral sides into ridges. The number of these ridges or folds in the section decreases as we pass from the median gill filament to those located either dorsally or ventrally on the septem

The endostyle (*en.*, figs. 1, 2, and 4) forms as a furrow in the floor of the pharynx (W. Müller, 1873; Scott, 1887; Stockard, 1906) and in the stage which we are considering extends under the first four gill pouches. Unlike a similar structure in Amphioxus, it does not communicate with the pharynx throughout its whole extent, but is roofed over both anteriorly and posteriorly, leaving a single slit-like connection (*en.*, fig. 2) with the pharynx in the segment through the third gill pouch.
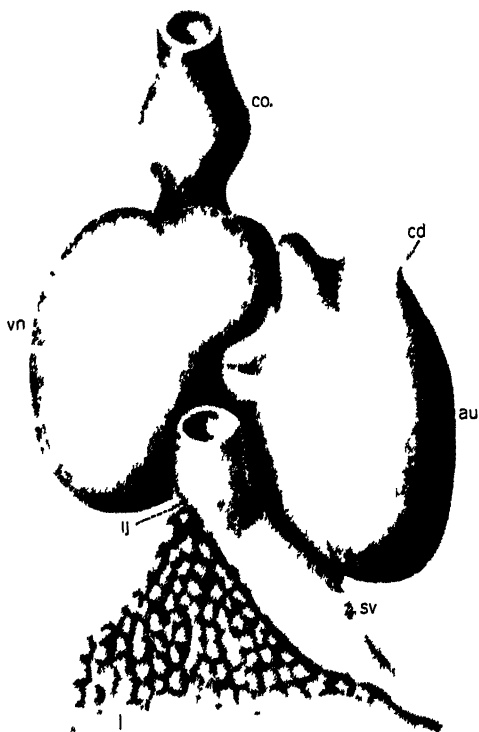

Fig. 3. Heart, 10 mm., Ammocoetes, ventral view.

au., auricle; *cd.*, cord from auricle to pericardium; *co.*, conus arteriosus; *ij.*, inferior jugular vein; *l.*, liver (anterior tip); *sv.*, sinus venosus; *vn.*, ventricle.

A transverse section through the anterior part of the endostyle shows four columns of cuneiform, secreting cells on a side (*sc.*, fig. 4); two of these columns, a larger upper and a smaller lower, empty laterally, and two, similarly a larger upper and a smaller lower, empty medianly into the canal or common chamber (*ca.*). If the anterior

chamber be followed posteriorly it will be found to be continuous with the slit-like connection (*cn.*, fig. 2), so that the secretion from it could enter the pharynx. Moreover, it could then pass forward through the grooves in the floor of the pharynx and be carried forward and upward along the ciliated peripharyngeal grooves (*pg.*, fig. 2) and then back to the oesophagus.

Had the section been taken some distance behind the slit-like connection the picture of these columns of cuneiform cells would have been complicated by the fact that the median column in the area of the fourth branchial pouch bends upward and then extends forward; it then bends downward and again runs backward.

Marine (1913) has shown for Ammocoetes of the brook lamphrey that the cuneiform cells degenerate at the time of metamorphosis, and that the follicles of the thyroid gland form from certain areas in the walls surrounding these columns.

C. The Trunk Area

Just posterior to the pharynx there are three prominent organs: the heart, the pronephros, and the liver.

The heart (fig. 3) seen in ventral view in the living organism is less compact than in higher vertebrates at a similar stage. It consists of the two main rooms, the auricle (*au.*) and the ventricle (*vn.*). Blood enters the auricle from the obliquely placed, thin-walled sinus venosus (*sv.*), passes through the auriculoventricular valves into the ventricle, and leaves the ventricle through the bulbus or conus arteriosus (*co.*).

In a ventral view of a living larva a slender cord (*cd.*) can be seen attaching the auricle to the pericardial wall. When the auricle is in systole this cord becomes taut; in auricular diastole it becomes loose again.

The pronephros is composed of four (or five) tubules (*pr.*, fig. 1) on a side, which may be seen just above the heart. Each tubule begins with a ciliated funnel which hangs free in the pericardial cavity. The tubules themselves can be followed a considerable distance in the living specimen before they become coiled, forming the main mass of the pronephros. Individually, these tubules finally enter the pronephrotic dust (*pd.*, fig. 5) which runs posteriorly and empties into the cloaca.
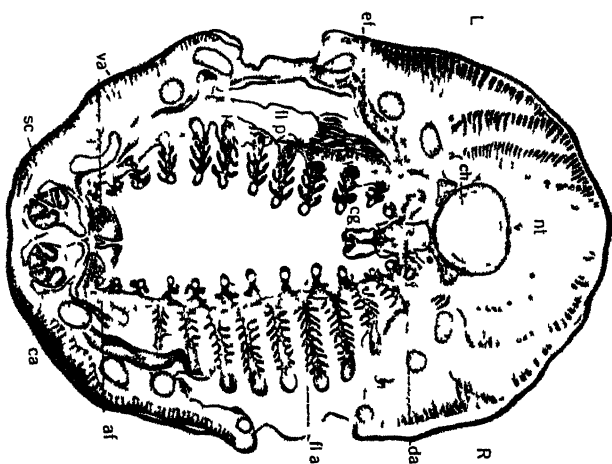
Fig. 4. Transverse section through pharynx, older larva, Ammocoetes.

Fig. 5. Transverse section through trunk, older larva, Ammocoetes.

af., afferent artery; *ca.*, canal of endostyle; *cg.*, ciliated dorsal groove; *ch.*, notochord; *da.*, dorsal aorta; *ef.*, efferent artery; *fd.*, fin fold; *fl.a.* and *fl.p.*, anterior and posterior gill filaments, respectively; *gn.*, gonad; *L*, left side; *nt.*, neural tube; *pc.v.*, postcardinal vein; *pd.*, pronephrotic duct; *R*, right side; *sc.*, secreting cells of endostyle; *s.i.v.*, subintestinal vein; *v.*, spiral valve; *va.*, paired ventral aorta.

The liver (*l.*, figs. 1 and 3) is relatively large in Ammocoetes. Seen in ventral view its anterior end is pointed, with the sinus venosus (*sv.*) fitting close to its margin. Posteriorly it is rounded off on the left side. In the living larva the hepatic cells making up the organ can be seen as individual units and groups, among which the circulating blood corpuscles form a remarkable picture. Located on the right side and more or less centrally among these cells is the relatively immense gall bladder (*gb.*, fig. 1).

The intestine extends from the posterior end of the pharynx to the anal opening and is divided into three segments, a narrow anterior oesophagus (*oe.*, fig. 1, Shipley, 1887), a median valvular part (*vi.*, fig. 1), and a postvalvular segment (*pv.*), all of which can be seen to advantage in side view of the trunk area. The narrow and most anterior segment, the oesophagus (*oe.*) passes from the posterior part of the pharynx backward and slightly upward the entire length of the liver. At the place where the oesophagus joins the valvular segment its lumen is convoluted and valve-like. The lining of the oesophagus is in part ciliated and these ciliated areas may be traced forward into the ciliated bands of the pharynx.

The median valvular segment of the intestine (*vi.*, fig. 1), containing the so-called spiral valve (*v.*, fig. 5), is the most conspicuous part of the tract, exceeding several times the diameter of the oesophagus. The valve is produced by a folding of the wall of the intestine obliquely inward from the ventrolateral side. Into this fold a tongue of connective tissue is formed in which the subintestinal vein (*si.v.*) runs.

Back of the valvular segment the postvalvular part (*pv.*, fig. 1) gradually decreases in diameter as it near the cloaca. The lining of both the valvular and the postvalvular intestine is ciliated in longitudinal bands continuous with those of the oesophagus.

In nature the valvular part of the intestine is usually filled with multitudes of diatoms (see fig. 5). It would appear that these serve as food, for in a living specimen, if the action of the intestine be studied and the course of the food followed, it will be observed that as it approaches the terminus of the postvalvular segment only débris is ejected through the cloaca. Miss Alcock (1899) has shown, however, that this segment of the tract is largely absorptive and that the greater part of digestion, at least of protein digestion, takes place in the pharynx.

D. THE TAIL

The tail represents the postcloacal segment of the body in which the notochord, neural tube, and myotomes terminate. Surrounding its margin is a fold (*fd.*, figs. 1 and 5) which is a part of the active locomotor propelling organ, the caudal fin. In the larval Ammocoetes there are no lateral folds like the metapleural folds in Amphioxus, parts of which are taken to be the homologues of paired appendages in higher chordates.

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CILIATES FROM BOS INDICUS LINN.

II. A REVISION OF DIPLODINIUM SCHUBERG

BY

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CONTENTS

INTRODUCTION

The original genera of the Ophryoscolecidae, *Entodinium, Diplodinium*, and *Ophryoscolex*, have been split up continually and recombined, as the knowledge of their morphology has been increased. Lack of agreement as to what constitutes valid generic characters, lack of designated type species, and incorrect revision of genera such as pointed out by Becker and Hsiung (in Hegner and Andrews, 1930), have resulted in considerable confusion, particularly in regard to *Diplodinium*. It is the purpose of this paper to bring order in place of this confusion, in so far as possible, in regard to the various genera and subgenera resulting from past revisions of *Diplodinium*.

MATERIAL AND METHODS

In the present investigation the same collections and material were used and the same procedure followed as were employed in our earlier paper (Kofoid and MacLennan, 1930). Unstained glycerine mounts were found to be most useful in dealing with species of large size. Whole mounts stained in haematoxylin were used mainly for checking results from the glycerine mounts. The system of proportional measurements previously described is used here also. It must be emphasized that these measurements apply only to fixed specimens. The contraction due to fixation produces a greater curvature of the

spines and obscures the lateral compression, as compared with living specimens. However, in examining material, both living and fixed, from California cattle, it was found that this distortion is relatively small and is fairly constant for a given species.

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REVIEW OF LITERATURE

The genus *Diplodinium* was established by Schuberg (1888) to include those species of Ophryoscolecidae having a short dorsal membranelle zone in addition to the adoral membranelle zone. Crawley (1923) set up the genus *Epidinium*, including in it species with the dorsal zone located considerably behind the level of the adoral zone, thus segregating Sharp's *Diplodinium ecaudatum* from *Diplodinium* s. str. Awerinzew and Mutafowa (1914) described the genus *Metadinium*, which, however, recent authors (Buisson 1923, Dogiel 1927) have considered unjustified. Buisson (1923) gave *D. bursa* as the type species of *Diplodinium*. *Diplodinium* was further revised by Dogiel (1927), who divided it into four subgenera: *Anoplodinium*, *Eudiplodinium*, *Polyplastron*, and *Ostracodinium*, without including *Diplodinium* as one of the subgenera.

TYPE SPECIES OF *Diplodinium* SCHUBERG

Stein (1858) established the genus *Entodinium*, including in it species of ciliates from cattle with an anterior rotatory organelle ("Wirbelorgan"), but which lack the girdle of cilia (present in *Ophryoscolex*) around the middle of the body. To these characteristics he adds the following general description: the anus lies on the posterior end; the nucleus is band-like, with micronucleus

("nucleolus") lying lateral to it; there are usually two contractile vacuoles—one anterior and one posterior. Three species are described, but none are figured: (1) *E. bursa* Stein with the body rounded posteriorly, and with a more or less prominent cleft in the posterior end containing the anus; (2) *E. dentatum* Stein, with six, inturned, styloid spines on the posterior end; (3) *E. caudatum* Stein, with three spines present, on one side a long, thin spine, on the other side, two tooth-like spines.

In regard to the arrangement of the cilia of *Entodinium*, Stein only states that all the motor organelles are concentrated at the anterior end of the body, and that there is no equatorial girdle of cilia as in *Ophryoscolex*. He states definitely that there are usually two contractile vacuoles present. In those Ophryoscolecidae with only one membranelle zone, i.e., *Entodinium*, as restricted by Schuberg (1888) and later authors, there is only one contractile vacuole present. This holds for the fifty-one species of this genus described up to 1930 (Kofoid and MacLennan 1930). In all species with two vacuoles, the anterior crown of membranelles is divided into two parts, an adoral spiral and a dorsal semicircle, placed closed together at the anterior end of the body. Therefore, from the evidence afforded by Stein's own observations, it is highly probable, if not certain, that his *Entodinium* included forms in which the "Wirbelorgan" is composed of a single adoral spiral of membranelles and also forms in which it is composed of an adoral spiral together with a closely associated dorsal semicircle of membranelles.

Schuberg (1888) described and figured for the first time Stein's *E. bursa* and *E. caudatum* along with a new species *E. minimum*. He showed that in these species all the cilia are arranged in a single adoral spiral. In the introduction to his discussion, he states that *E. dentatum* and several ciliates which Stein had included with *E. bursa*, differed from his (Schuberg's) other three species of *Entodinium* in that a dorsal semicircle of cilia was present close to the oral spiral.

Eberlein (1895) disputed the existence of the two membranelle zones reported by Schuberg in Stein's *E. dentatum* and claimed to have found only an adoral spiral in this species. Since none of the many later workers has corroborated Eberlein's findings, but many times have found ciliates corresponding to Schuberg's description, we feel that Eberlein was mistaken, and that Stein's *E. dentatum* and Schubergs *Diplodinium dentatum* are identical.

Schuberg placed *E. dentatum* Stein and the other unnamed ciliates with two anterior membranelle zones in a new genus *Diplodinium*. He intended to publish a full description of *Diplodinium* and its species in a second paper, but this was never done.

Since *D. dentatum* was the only species of the genus when it was established, it is the type species. In spite of the fact that it had never been figured by either Stein or Schuberg, their description of the six, incurved caudal spines makes it possible absolutely to identify the species. Buisson (1923) gives *D. bursa* as the type species of *Diplodinium*. This species was not in the genus as originally described, however, and so cannot stand.

Fiorentini (1889) published the first figures of any species of *Diplodinium* and he has been widely quoted. Unfortunately, he ignored Schuberg's description of *D. dentatum*, thereby causing a great deal of confusion, and described this species as *D. denticulatum*, using the name *D. dentatum* (ascribing the name to himself) for a species possessing three broad caudal lobes. Railliet (1890), Awerinzew and Mutafowa (1914) drew attention to this confusion and corrected the mistake. Railliet, the first reviser, restored the name *E. dentatum* to the six-spined species, and renamed Fiorentini's *D. dentatum* as *D. mammosum* (Dogiel's *Ostracodinium dentatum*). Sharp (1914) showed that *D. dentatum* is the type species of *Diplodinium*, but recommended that Fiorentini's changes be retained. Fiorentini, however, did not emend Schuberg's descriptions, but changed the name of the six-spined species and treated his *D. denticulatum* (Schuberg's *D. dentatum*) in his descriptions as a new species.

TABLE SUMMARIZING THE HISTORY OF
Diplodinium dentatum and *Ostracodinium mammosum*

Author	Six-spined species	Three-spined species
Stein 1858	*Entodinium dentatum*	———
Schuberg 1888	*Diplodinium dentatum*	———
Fiorentini 1889	*Diplodinium denticulatum*	*Diplodinium dentatum*
Railliet 1890	*Diplodinium dentatum*	*Diplodinium mammosum*
Eberlein 1895	*Diplodinium dentatum*	
Awerinzew and Mutafowa 1914	*Diplodinium denticulatum*	*Diplodinium fiorentinii*
Buisson 1923	*Diplodinium dentatum* var. *denticulatum*	*Diplodinium dentatum*
Dogiel 1927	*Anoplodinium denticulatum* forma *denticulatum*	*Ostracodinium dentatum*
Name adopted by us	*Diplodinium dentatum*	*Ostracodinium mammosum*

Because of these changes by Fiorentini without regard for the law of priority, *D. dentatum* (Stein) Schuberg is retained as the name of the six-spined *Diplodinium* (Dogiel's *Anoplodinium denticulatum denticulatum*); the name *D. dentatum* Fiorentini, referring to the species with three caudal lobes (Dogiel's *Ostracodinium dentatum*), is preoccupied by Schuberg's species and must be rejected and *Ostracodinium mammosum* (Railliet) Dogiel substituted in its place.

REVISION OF *Diplodinium* SCHUBERG

Dogiel (1927) established four subgenera of *Diplodinium: Anoplodinium, Eudiplodinium, Ostracodinium,* and *Polyplastron.* Becker and Hsiung 1930 (in Hegner and Andrews 1930, p. 50) drew attention to this revision: "The *International Rules of Zoological Nomenclature,* Art. 9, states: 'If a genus is divided into subgenera, the name of the typical subgenus must be the same as the name of the genus.' Dogiel has disregarded this rule; hence a correction will be necessary here." Since the type species *D. dentatum* falls within the subgenus *Anoplodinium,* the name *Anoplodinium* is a synonym of *Diplodinium,* the true name of the typical subgenus.

The four subgenera established by Dogiel show important differences in nuclear structure and skeletal plates, which distinctly separate them. We therefore raise these subgenera to full generic rank and, in addition, reestablish the genus *Metadinium* Awerinzew and Mutafowa 1914, which was suppressed by Dogiel. Two distinct groups of *Diplodinium* s. str. were found in this study on the species from *Bos indicus,* and on this basis, *Diplodinium* is further restricted, and a new genus *Eodinium* is described. *Eudiplodinium* has been restricted and a new genus *Eremoplastron* erected. Dogiel's original description of *Polyplastron* has been retained and the species described by him later (Dogiel 1928) has been put in a new genus *Elytroplastron. Ostracodinium* Dogiel has been restricted and the genus *Enoploplastron* erected.

GENERIC CHARACTERS WITHIN THE OPHRYOSCOLECIDAE

In the earlier descriptions of *Diplodinium* (Schuberg 1888, Fiorentini 1889, Eberlein 1895) the possession of a short dorsal membranelle zone in addition to the adoral zone was the only generic character given. Crawley (1923) showed that this description alone was too inclusive and separated the forms with a short dorsal zone parallel to the adoral zone (*Diplodinium*) from those with the dorsal zone located farther back on the body (*Epidinium*). This was largely the result of the careful morphological work by Sharp (1914). As the knowledge of the morphology of the whole group has advanced, systematists have given more closely drawn generic characters and have tended to emphasize internal structures as well. Dogiel, in his monograph on the Ophryoscolecidae (1927), has made use of the skeletal plates, macronuclei, and contractile vacuoles in his descriptions of genera. This use of the important internal structures, as well as of the external structures, is a great advance toward a natural classification of the Ophryoscolecidae, as well as a great help in the practical recognition of genera and species. The confusion of identification which often results from descriptions based entirely on external features has been clearly pointed out by Jameson (1925).

The skeletal plates are very constant, highly characteristic structures. They vary from one to five in number; when only one is present, it is either very narrow or very broad; when more than one is present the width is usually medium. Each type of skeletal system is constant over a number of species, and correlated with these differences in skeletal plates may be found differences in macronuclear structure, thickness of ectoplasm, size of rectum, and number of contractile vacuoles. This has led us to a greater emphasis on the internal structures, and a restriction of each genus to species which are similar in all the above characters. These morphological features are easily found in either living or fixed material, are discontinuous and easily distinguished, and are independent of variations in size and proportions. We believe, therefore, that the use of these internal morphological characters, in addition to the use of the morphology of the membranelle zones, will lead to a more natural classification of the Ophryoscolecidae and at the same time will greatly facilitate practical identification of these ciliates.

OCCURRENCE OF GENERA AND SPECIES IN *Bos Indicus*

The same method of counting followed in the first paper of this series was used to obtain the following data. The ciliates considered in these counts were the Ophryoscolecidae exclusive of the genus Entodinium. The columns are numbered to correspond to the numbers given to the original collections. In all, nine hosts were examined.

Bos indicus	Coonoor, India					Colombo, Ceylon				Number hosts infected
	1	2	3	16	17	6	7	14	15	
Eodinium lobatum ...		P*							6	P	3
polygonale	1					P	P	1		4
Diplodinium dentatum	56	73	26	33	17	59	42	18		8
monacanthum	2					P	P	1	3	5
psittaceum	10	P	8	P	9	P		3		7
flabellum	1		2		7					3
Eremoplastron rostratum						P	P			2
rotundum	6	P	13	59	27		5	7	5	8
bovis			1	2	3		14	11	32	6
brevispinum									P	1
magnodentatum			1			P				2
Eudiplodinium maggii	17	6	15	1	9	21	15	6	15	9
Metadinium medium	7	7	7	P	4	7	6	18	5	9
Ostracodinium mammosum			4		14	P	5	2		5
gracile		10	4	1	4		11	7	11	7
trivesiculatum			8	1	4		P	P	8	6
quadrivesiculatum			1	P				2	5	4
clipeolum	P	4	6	3		P		2	P	7
rugoloricatum			1						1	2
venustum			P		1		P		2	4
Elytroplastron bubali			1		P		P	5		4
Epidinium			2	P	1	13	2	10	13	7
Ophryoscolex			P		P					2

* The numbers in the columns indicate the percentage occurrence of individuals of each species. P indicates that the species is present, but in such small numbers that it was not found during the counting.

The distribution of the above species shows no real difference between the ciliate fauna of the Ceylon type and of the Indian type of *Bos indicus*. This distribution agrees with that of the species of *Entodinium* in the same hosts.

GENERAL MORPHOLOGY OF *EODINIUM* GEN. NOV. AND *DIPLODINIUM* S. STR.

The morphology of the Ophryoscolecidae shows few basic differences from genus to genus. The differences consist mainly of an increase in the number of homologous parts and a change of proportion in various structures. The range from one contractile vacuole in *Entodinium* to fifteen in *Ophryoscolex* illustrates the first class of difference; the change in length and position of the dorsal membranelle zone from a short, anteriorly located zone in *Diplodinium*, through a series to the long, medianly placed zone in *Caloscolex*, illustrates the second class. Only a few structures found in the more complex genera are entirely lacking in the simpler genera. A dorsal membranelle zone is lacking in *Entodinium* alone. Skeletal plates are lacking in *Entodinium*, *Eodinium*, and *Diplodinium*. Because of this essential similarity in structure, only those points in which *Eodinium* and *Diplodinium* differ from the other genera will be treated in detail.

Since both *Eodinium* and *Diplodinium* lack skeletal plates and are similar in general morphology, it is convenient to treat the morphology of both genera together.

The proportions, shape of surfaces, and presence of surface striations in *Diplodinium* and *Eodinium* are similar to those described for *Entodinium*. The genus *Eodinium*, with an average length of 48μ, shows no advance in size over *Entodinium*, but the species of *Diplodinium* average considerably larger, being about 100μ in length; Dogiel reporting individuals up to 210μ in length. The average size of species of *Entodinium* is about 45μ, the largest individuals being only about 120μ.

The caudal structures of *Diplodinium* show an advanced stage of evolution in having a unique and complex type of tail in addition to the various combinations of spines, lobes, and flanges found in *Entodinium*. This complex caudal structure occurs in *Diplodinium cristagalli* and in *D. flabellum*. It is a flat, fan-like structure projecting posteriorly from the right surface, in a parasagittal plane on the right of the anus. It bears from two to seven radiating teeth on the posterior margin. Both the position and shape of this tail differ from those found in the other Ophryoscolecidae. The number of radiating

spines shows considerable variation. Dogiel (1927) reports in *D. crista-galli* a variation of from two to six spines. We have found in *D. flabellum* a variation of from five to seven spines (fig. D, 5–8) with small secondary spines often appearing between the main spines. The main spines are often bifurcate or trifurcate. The series given by Dogiel indicates the evolution of this fan of spines from a simple flattened lobe.

The caudal structures of the reported species of *Eodinium* consist of only one or two lobes. This simplicity is a marked contrast to the complexity of the tails found in most of the other genera of Ophryoscolecidae.

The presence of a dorsal membranelle zone in addition to the oral zone characteristic of all Ophryoscolecidae sharply separates the genera *Diplodinium* and *Eodinium* from *Entodinium*. The oral zone is similar in all respects to that described by Sharp (1914) in *Epidinium* (his *Diplodinium*), and by us in *Entodinium* (Kofoid and MacLennan 1930). The dorsal zone consists of from ten to twenty membranelles arising in a transverse furrow on the dorsal surface of the body. This zone is at the same level of the body as the oral zone. The furrow extends for one-half to one-third of the circumference around the anterior end of the body. There are two concentric, ectoplasmic lips bounding the outer surface of the dorsal zone. The outer dorsal lip (fig. A, *out. d. lip*) homologous to the outer adoral lip (fig. A, *out. ad. lip*), is the anterior termination of the dorsal surface of the body. A deep groove, the outer dorsal furrow (fig. A, *out. d. fur.*), lies between the outer adoral lip and the relatively thin, inner dorsal lip (fig. A, *in. d. lip*). The inner dorsal lip lies close against the dorsal membranelles. A rounded, ectoplasmic projection, the dorsal disk (fig. A, *d. disk*), lies in the semicircle bounded by the dorsal membranelles. The dorsal disk is greatly obscured in species with a large inner dorsal lip.

Each membranelle, in life, is a thin, apparently homogeneous bundle of cilia, tapering to a point at its distal end. In material from *Bos indicus* fixed in Schaudinn's fluid, the individual cilia have largely separated and only faint traces of the original compact structure are left, most of the membranelles being completely frayed out into their component cilia.

The two membranelle zones are separated by an ectoplasmic elevation, the operculum (fig. A, *oper.*). This structure is formed by an

apical continuation of the lateral body surfaces. In specimens with retracted membranelle zones, this operculum is very prominent.

The outer grooves of both membranelle zones are connected in some species by a shallow groove extending along the base of the operculum (fig. A). In some species it occurs on one side only, in some on both, but in the great majority of species it is absent.
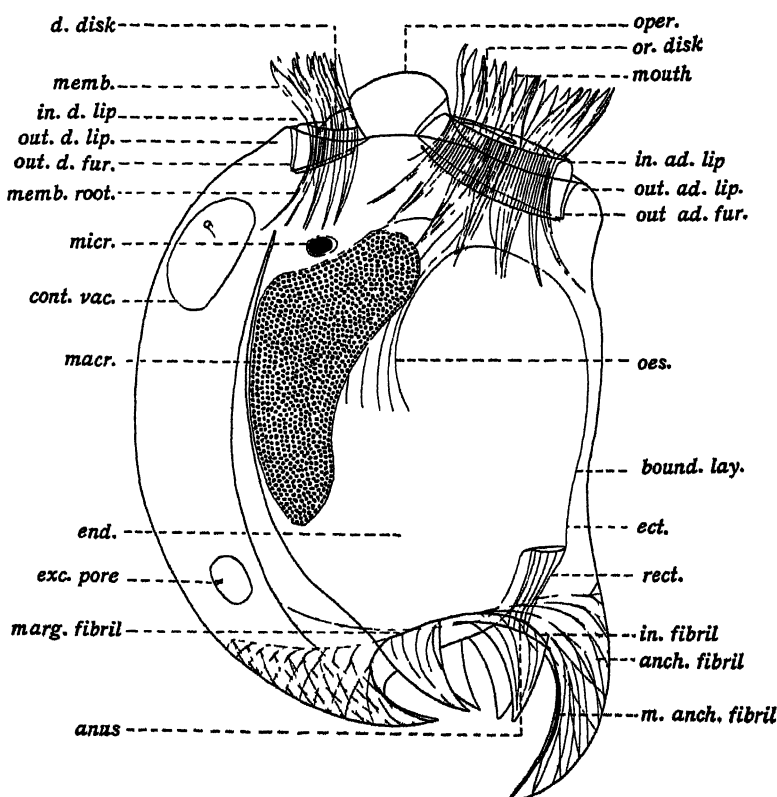
Fig. A. *Diplodinium dentatum* (Stein) Schuberg. Semi-diagrammatic lateral view. The surface striations are omitted for the sake of clearness. ×1000. *anch. fibril*, anchoring fibril; *anus*, anus; *bound. lay.*, boundary layer; *cont. vac.*, contractile vacuole; *d. disk*, dorsal disk; *ect.*, ectoplasm; *end.*, endoplasm; *exc. pore*, excretory pore; *in. ad. lip*, inner adoral lip; *in. d. lip*, inner dorsal lip; *in. fibril*, inner fibril; *macr.*, macronucleus; *m. anch. fibril*, main anchoring fibril; *marg. fibril*, marginal fibril; *memb.*, membranelle; *memb. root*, membranelle root; *micr.*, micronucleus; *mouth*, mouth; *oes.*, oesophagus; *oper.*, operculum; *or. disk*, oral disk; *out. ad. fur.*, outer adoral furrow; *out. ad. lip*, outer adoral lip; *out. d. fur.*, outer dorsal furrow; *out. d. lip*, outer dorsal lip; *rect.*, rectum.

The locomotor apparatus of the more complex genera of Ophryoscolecidae, such as *Epidinium* or *Caloscolex*, shows no fundamental

advance over the locomotor apparatus as it appears in *Eodinium* and *Diplodinium*. The relative size and position of the two zones vary from genus to genus, but no fundamental additions are made. The intraplasmic fibrils of the neuromotor apparatus have not been the object of particular interest in this investigation, but those parts which have been incidentally observed correspond exactly to Sharp's (1914) description of the neuromotor apparatus in *Epidinium* (his *Diplodinium*).

A complex, fibrillar net occurs in the caudal spines of *Diplodinium*, in direct contrast to the simple, apparently structureless spines in *Entodinium*. The caudal fibrils of *D. dentatum* furnish a relatively complex example of this type of caudal fibrillar system.

A heavy marginal fibril (fig. A, *marg. fibril*) extends along the bases of the spines on each side of the body, terminating in the dorsal and ventral spines. Finer anchoring fibrils (fig. A, *anch. fibril*) extend into each spine from the main marginal fibril and fasten under the cuticle of the outer margin of the spines. An exceptionally heavy anchoring fibril, the main anchoring fibril (fig. A, *m. anch. fibril*), extends under the inner edge of each spine. Smaller fibrils (fig. A, *in. fibril*) arise from the anchoring fibrils of the ventral spine and extend a short distance dorsally, one on each side of the anus. Small branches given off from these fibrils lie in the wall of the rectum, parallel to its main axis.

This caudal fibrillar system shows no apparent association with the locomotor organs, or with their fibrils. They therefore do not seem to be a part of the neuromotor system. Their location and relationships suggest a general supporting and reinforcing function, similar to that of the longitudinal surface fibrils. In addition, the main anchoring fibrils, together with the marginal fibrils, are admirably situated to produce a change of curvature in the spines. In living specimens from California cattle, it was observed that the curvature of the spines in an individual may vary from time to time. In material fixed with Schaudinn's fluid, the spines are always bent inward. This evidence indicates the presence of contractile elements along the inner surface of the spines, functional in life and also more strongly contracted by fixatives than the rest of the ectoplasm. We therefore consider these caudal fibrils as myonemes.

Bělar (in Hartmann, 1925, p. 101, fig. 54) demonstrated a similar fibrillar system in *Epidinium caudatum*, and Dogiel (1926) an even more complex system in *Caloscolex camelinus*. On the basis of Bělar's

investigation, Reichenow (1929) considers the neuromotor function of all fibrils of the Ophryoscolecidae to be disproved. The morphological relationships, however, show the caudal fibrils and the neuromotor fibrils to have entirely different functions.

1. The caudal fibrils are admirably situated to serve as supporting and contractile structures. The motor fibrils are so situated as to be of little or no use either as supporting or contractile fibrils.

2. The caudal fibrils show no connection to the motor organelles. The motor fibrils link together all the motor organelles of the individual.

3. The caudal fibrils are not connected to a large central mass, they are merely an interconnecting network. The motor fibrils, on the other hand, converge upon the large central mass, termed the neuromotorium by Sharp (1914).

The clear difference between the relationships of the caudal fibrils and the neuromotor fibrils emphasizes the strength of the morphological evidence for the neuromotor hypothesis.

The mouth opens into a short, ectoplasmic tube, the gullet, which continues into the endoplasm and terminates in the midregion of the body. This latter section of the tube is the oesophagus. The wall of the gullet and oesophagus is made up of thin, closely spaced fibrils extending parallel to the long axis of the tube. There is usually a slight enlargement of the oesophagus where it passes through the boundary layer; the rest of the tube varies very little in diameter. The oesophagus extends into the endoplasm dorsally and to the right at an angle of about 45° with the main axis. No striking variation in the form of the oesophagus, such as occurs in *Entodinium,* was found in either *Eodinium* or *Diplodinium.*

The boundary layer, a thin, ectoplasmic membrane, separates the ectoplasm from the endoplasm, as in the other Ophryoscolecidae. The boundary layer in *Eodinium* is thin and seen with difficulty, but in *Diplodinium* it is usually an obvious and well developed membrane. The endoplasmic sack occupies the main part of the body, arising just behind the oral membranelle zone and terminating posteriorly at the rectum.

The rectum is a thin-walled tube arising ventrally from the endoplasmic sack, extending posteriorly through the ectoplasm and opening to the exterior by a permanent opening, the anus. Rectal fibrils have been found in a few species. Longitudinal fibrils line the rectal wall of *Diplodinium dentatum.* Dogiel (1927) reports trans-

verse circular myonemes in *Diplodinium psittaceum.* The rectum is usually flattened dorso-ventrally. It lies in the main sagittal plane in most species and extends dorsally at an angle of about 45°.

The rectum of *Diplodinium flabellum* (fig. D, *3–4*) is unique in all particulars. It is circular in cross-section, with the smallest diameter at its anterior end. It flares toward the posterior end and terminates in a large, circular anus. The rectum arises on the right side of the posterior end of the endoplasmic sack and extends dorsally and to the mid-line, at an angle of 40°–50°.

The rectum in the species of *Eodinium* is short, only slightly flattened, and thin-walled. On the other hand, in *Diplodinium,* the rectum is relatively larger, heavy-walled, and in some species, highly specialized in form.

The position of the macronucleus divides the species of Dogiel's subgenus *Anoplodinium* into two distinct groups, one with the macronucleus located dorsally, the other with the macronucleus located near the middle of the right surface. Corresponding to this difference in position is a difference in shape. In the latter type of macronucleus which Dogiel notes as "typical," the anterior third of the macronucleus is bent ventrally at an angle of 45°–50° with the posterior two-thirds of the dorsal side. In species with a short, heavy macronucleus, this flexure produces a triangular shape (pl. 4, fig. 5), in species with elongate macronuclei, a boomerang shape (fig. B, 5). A study of the division of the macronucleus of *Diplodinium dentatum* emphasizes the stability of the shape of the macronucleus. The macronucleus begins to elongate and constrict in the middle after the telophase of the micronuclear division is reached. The posterior part, destined to become the macronucleus of the posterior daughter, begins to assume the bend and shape of the nucleus of the parent organism at the very outset of macronuclear division (fig. B, 7). The normal shape is fully attained by the time the daughter organisms separate. The persistence of the characteristic angle in spite of a wide variation in the size and proportions of the macronucleus of individuals of a species and between different species, and even during division stages, marks it as a character of systematic importance. The macronucleus occurring in those species in which the macronucleus is dorsally located is a narrow and elongated type. It is usually straight, but it may be curved slightly laterally, to conform to the curvature of the lateral surface of the body. This type never shows a ventral bend.

The micronucleus is a small, ellipsoidal body, from three to eight μ in diameter. An apparently homogeneous mass of chromatin is separated from the thin nuclear membrane by a narrow, clear region. The micronucleus is similar in structural features to that found in the rest of the Ophryoscolecidae.

The contractile vacuoles have the same form as in *Entodinium*. They are simple, lentoid vesicles, facing dorsally and opening to the exterior by a short excretory canal and pore. There are always two vacuoles (with the possible exception of *Diplodinium flabellum*), both placed near the dorsal mid-line. In the *Eodinium posterovesiculatum* group, the vacuoles lie in depressions in each end of the macronucleus, but in all other species of both genera they show no morphological relationship to the macronucleus.

FOOD

The food particles observed within the species of *Diplodinium* were bacteria, flagellates, and plant débris of relatively large size. The species of *Eodinium* contained mainly flagellates and bacteria, with occasional small bits of plant material. No restricted food preferences such as occur in *Entodinium* were found in the above two genera.

The above review of the general morphology of the species included in Dogiel's subgenus *Anoplodinium* shows that those species fall into two groups, separated not only by rather obvious differences in the shape and position of the macronucleus, but also by correlated differences in size and in complexity of parts. We give these two groups generic rank as *Diplodinium* and *Eodinium*. Within these genera, the species fall into natural groups composed of species very closely allied, and usually differing only in number and position of spines. With an increase in the number of known species of Ophryoscolecidae these groups may form the basis of accurately defined subgenera, but until a much larger proportion of the possible hosts of the family have been investigated, this would be unjustifiable.

The policy of eliminating the systematic rank of "forma" adopted in the first paper of this series has been followed in the present paper. All the available evidence indicates that the number of spines and other morphological differences which are the chief distinguishing features of "formas" are stable and unchanging from generation to generation. An interesting bit of experimental evidence on the gen-

eral question of the validity of the genera, species, and formas of the Ophryoscolecidae has been furnished by Becker, Schulz, and Emmerson (1930). They state:

Becker and Talbott (1927) were inclined to the belief, implied in several places in their paper, that the different form types of Ophryoscolecidae did not necessarily represent species or even varieties, and that they might be merely transitional forms. Consequently, they adopted a policy of "lumping" as contrasted with Dogiel's policy of "splitting." We now feel that Dogiel has adopted the correct course, for our experience has taught us that when a defaunated animal is inoculated with certain known types, the ensuing fauna developing in its rumen do not offer any striking modifications of the original types.

Eodinium gen. nov.

Anoplodinium Dogiel, *partim*, 1927, pp. 75–77, figs 37–39, (for pp. 77–119, figs. 40–66, see *Diplodinium*).

Diagnosis.—Ophryoscolecidae with dorsal membranelle zone on the same level as the adoral zone; no skeletal plates; macronucleus a straight, rod-like body beneath the dorsal surface of the body; two contractile vacuoles present.

Type.—*Eodinium lobatum*, sp. nov. from *Bos indicus* from Colombo, Ceylon.

Eodinium is composed of a number of species of Dogiel's subgenus *Anoplodinium* which are related to the "typical" species of that group only by a single characteristic, the lack of skeletal plates. The position and shape of the macronucleus in this genus, as shown in the above discussion of its morphology, clearly separates it from the other species of Dogiel's *Anoplodinium*. In addition, the relatively small operculum, simplicity of caudal armature, and weak development of the endoplasmic sack and rectum mark it off from *Anoplodinium*. The range in size is markedly different in the two genera. Combining the data from Dogiel's monograph and from this paper, the species of *Eodinium* average 48μ in length with a size range of from 32 to 60μ; the species of *Diplodinium*, on the other hand, average 100μ, with a range from 55 to 210μ.

POSTEROVESICULATUM GROUP—

The *Posterovesiculatum* group of *Eodinium* is distinguished by the close relationship between the macronucleus and the contractile vacuoles. The macronucleus is long and narrow. The anterior vacuole lies close against the left side of its anterior end; the posterior vacuole lies directly behind the posterior end of the macronucleus. This group includes *Eodinium posterovesiculatum* (Dogiel 1927), *Eodinium lobatum* sp. nov., and *Eodinium bilobosum* (Dogiel 1927).

Eodinium posterovesiculatum (Dogiel 1927)

Anoplodinium posterovesioulatum forma *posterovesiculatum* Dogiel 1927, pp. 75–76, fig. 38*a*, *b*.
Diplodinium posterovesiculatum Dogiel 1927, p. 261.

Diagnosis.—Body relatively long (2.00 dorso-ventral diameters in length) and laterally compressed. Dorsal surface convex, ventral surface flat or slightly concave; posterior end smoothly rounded. Length 52μ (47–60μ).

Occurrence.—In domestic cattle from U.S.S.R. (Dogiel 1927) and from South Africa (Fantham 1926).

Eodinium lobatum sp. nov.

Plate 4, figure 3; figure B, 1, 2

Diagnosis.—Body small and narrow; the two vacuoles rest in depressions near each end of the macronucleus; a distinct ventral lobe. Length: 44–60μ, 10 specimens.
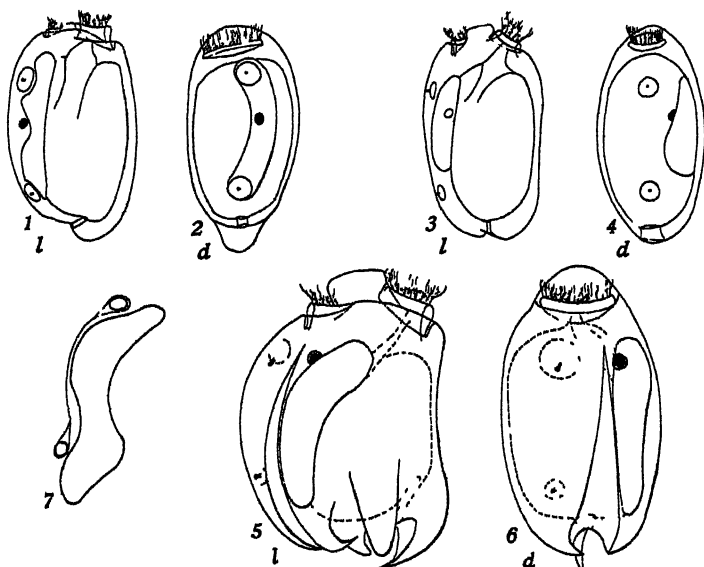
Fig. B.   1 and 2, *Eodinium lobatum* sp. nov.; 3 and 4, *E. rectangulatum* sp. nov.; 5 and 6, *Diplodinium dentatum* Schuberg 1888; 7, *Nuclei* of *D. dentatum* in late telophase.   *d*, dorsal view; *l*, lateral view.   × 500.

Description.—The body is a rather narrow ellipsoid (1.51–1.97 dorso-ventral diameters in length) and laterally compressed (0.86–0.93 dorso-ventral diameters).   The mouth is small in diameter (0.30–0.39 dorso-ventral diameters).   It is inclined ventrally at an angle of 10°–15° but is not inclined to either side.   The dorsal zone

and dorsal disk are small and inconspicuous. The dorsal membranelle zone is at an angle of about 10° with the main axis. The operculum is very small. The dorsal and ventral surfaces, in the middle half of the body, are only slightly convex and are nearly parallel. In the posterior quarter of the body the surfaces suddenly bend inward toward the anus. From a dorsal view the body is nearly a perfect ellipse, but the right side is slightly more convex than the left. There is a distinct ventral lobe (0.06–0.14 dorso-ventral diameters in length) occupying one-third of the dorso-ventral diameter of the posterior end.

The macronucleus is a narrow, rod-like body (1.00–1.33 dorso-ventral diameters in length), with three large depressions in its dorsal side. A depression slightly behind the anterior tip receives the anterior vacuole, the large one in the middle, the micronucleus, and the one in the tip of the posterior end receives the posterior vacuole. The ends of the macronucleus lie in the dorsal mid-line, but the nucleus as a whole is curved in an arc parallel to the right surface. The macronucleus is a small, ellipsoid body from 3 to 6μ in diameter.

The contractile vacuoles, lying on the two ends of the macronucleus, are slightly compressed dorso-ventrally. Each opens to the surface by a short canal and small excretory pore located near the dorsal mid-line.

The oesophagus is a slightly conical, tubular structure, indistinctly marked by a loose bundle of fibrils in its periphery. It ends near the middle of the macronucleus. The endoplasmic sac originates at the level of the anterior end of the macronucleus and extends posteriorly, closely following the outline of the body. The rectum is a short tubular structure extending dorsally from the posterior end of the endoplasmic sack at an angle of about 45°. It opens in the middle of the posterior surface through an elliptical anus.

Food.—Bacteria, small flagellates, and occasional small bits of plant material are found in the endoplasmic sack.

Variations.—There are no important variations in this species.

Measurements.—The following measurements were taken from ten individuals picked at random:

Axis	Microns	Proportional
Length	55 (44–60)	1.73 (1.51–1.97)
Transdiameter	28 (26–30)	0.90 (0.86–0.93)
Dorso-ventral diameter	32 (29–37)	1.00
Macronucleus	37 (29–40)	1.16 (1.00–1.33)
Mouth	11 (10–13)	0.34 (0.30–0.39)
Ventral lobe	4 (2– 6)	0.13 (0.06–0.14)

Occurrence.—*Eodinium lobatum* occurred in small numbers in one *Bos indicus* examined from Coonoor, India, and in two from Colombo, Ceylon.

Relationships.—*Eodinium lobatum* is similar to *Eodinium posterovesiculatum* (Dogiel 1927) in all respects except the absence of the ventral lobe in the latter species. *Eodinium posterovesiculatum* (Dogiel 1927), *Eodinium lobatum* sp. nov., and *Eodinium bilobosum* (Dogiel 1927) thus form a series showing the transition from no caudal projections to two caudal lobes.

Eodinium bilobosum (Dogiel 1927)

Anoplodinium posterovesiculatum forma *bilobosum* Dogiel 1927, pp. 76–77,
    fig. 39 *a, b.*

Diagnosis.—Body relatively short (1.40 dorso-ventral diameters in
length) and laterally compressed; dorsal surface convex, ventral sur-
face flat or slightly concave. Two caudal spines present, the dorsal
spine inclined to the right, the ventral spine to the left. Length 52µ
(46–60µ); dorso-ventral axis 36µ (30–41µ).

Occurrence.—In domestic cattle and sheep from Union of Socialist
Soviet Republics, Siberia, and Turkestan (Dogiel 1927).

UNALLOCATED SPECIES—

    This group includes *Eodinium polygonale* (Dogiel 1925) and
Eodinium rectangulatum sp. nov.

Eodinium polygonale (Dogiel 1925)

Diplodinium polygonale Dogiel 1925, p. 120, fig. 2c, *d*; 1927, p. 261;
    Fantham 1926, p. 527, fig. 6.
Anoplodinium polygonale Dogiel 1927, p. 75, fig. 31a, *b.*

Diagnosis.—Body small and short (1.6 dorso-ventral diameters in
length), roughly hexagonal in outline; dorsal surface flat, ventral
surface convex; no caudal projections. Macronucleus short and heavy,
approximately one-third of the length of the body in length. Length
35µ (32–38µ); dorso-ventral axis 22µ (20–24µ).

Occurrence.—Reported by Dogiel (1927) from the steenbuck
(*Rhaphiceros* sp.) from British East Africa.

Eodinium rectangulatum sp. nov.

Plate 4, figure 4; figure B, 3, 4

Diagnosis.—Body rectangular in lateral view, ellipsoidal in dorsal
view; macronucleus to right of mid-line; no caudal lobes. Length
35–70µ, 7 specimens.

Description.—The body is somewhat rectangular in side view
(1.63–1.95 dorso-ventral diameters in length). It is ellipsoidal from
a dorsal view and compressed laterally (0.85–0.94 dorso-ventral
diameters in transdiameter). The oral area is small in diameter (0.25–
0.33 dorso-ventral diameters) and inclined ventrally at an angle of
20°–45°. There is no lateral slope of the oral area. The dorsal zone
is small and inconspicuous. The dorsal disk is small. The operculum
is of relatively medium size. The dorsal surface has a weak convex
curvature, but the middle half of the ventral surface is slightly con-
cave. The lateral surfaces are distinctly convex. The posterior end
of the body is rather square in side view, with a slight groove in the
middle where the anus is located.

    The macronucleus is relatively short (0.56–0.79 dorso-ventral
diameters in length). The posterior end of the macronucleus is from

two to four times as large as the anterior end in both dimensions. The macronucleus is located on the dorsal surface to the right of the mid-line. Although the nucleus is located somewhat to the side of the dorsal surface, it displays no ventral curvature, and so is entirely distinct from the *Diplodinium* type of macronucleus. The micronucleus is located to the left of the middle part of the macronucleus, usually lying in a small depression. The micronucleus is a small, spherical body from three to four μ in diameter.
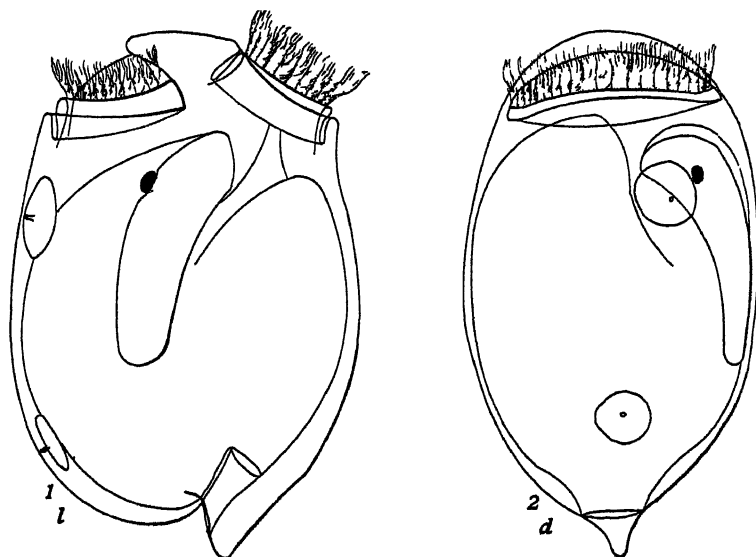
Fig. C. *Diplodinium psittaceum* Dogiel 1927, *d*, dorsal view; *l*, lateral view. × 500.

The two small contractile vacuoles are located on the dorsal mid-line. The anterior one is at the level of the anterior end of the macronucleus. The posterior vacuole is located somewhat behind the level of the posterior end of the macronucleus.

The oesophagus consists of a small, indistinct bundle of fibrils extending posteriorly into the endoplasm at an angle of about 30°. It ends near the mid-region of the macronucleus. The endoplasmic sack originates just behind the base of the oral area and extends posteriorly to near the end of the body. The boundary layer is quite distinct. The ectoplasmic layer is thick dorsally and in the caudal region, but thin ventrally and laterally. The rectum is a short, simple, slit-like structure. It extends from the middle of the posterior end of the endoplasmic sack, parallel to the main axis of the body. The rectum opens by the small elliptical anus located in the caudal groove.

Food.—The food consists of bacteria and small flagellates.

Variations.—The depth of the caudal groove varies to some extent, but it is never deep enough to separate the caudal ectoplasm into dorsal and ventral lobes.

Measurements.—The following measurements were taken from the seven specimens found, of which only three were expanded:

Axis	Microns	Proportional
Length	53 (35–70)	1.85 (1.63–1.95)
Transdiameter	26 (17–37)	0.90 (0.85–0.94)
Dorso-ventral diameter	29 (18–40)	1.00
Macronucleus	21 (12–30)	0.70 (0.56–0.79)
Mouth	8 (7–10)	0.28 (0.25–0.33)

Occurrence.—*Eodinium rectangulatum* was found in very small numbers in one host from Coonoor, India, and in three from Colombo, Ceylon.

Diplodinium Schuberg emended Crawley emended Dogiel emended

> *Diplodinium* Schuberg, 1888, p. 404; Crawley, *partim*, 1923, pp. 395, 400, pl. 18, fig. C2 (for pp. 400, 403, pl. 28, fig. B3, see *Anoplodinium;* for pp. 403, pl. 28, figs. B1–2, see *Eudiplodinium;* for pp. 403, 404, pl. 28, fig. B5, see *Eremoplastron*); Dogiel, *partim*, 1927, pp. 71–72, 77–105, figs. 40–56 (for pp. 120–121, figs. 37–39, see *Eodinium;*. for pp. 105–119, figs. 57–66, see *Eremoplastron;* for pp. 119–122, fig. 67*a, b*, see *Eudiplodinium;* for pp. 123–124, fig. 68, see *Enoploplastron;* for pp. 124–130, figs. 69–72, see *Metadinium;* for pp. 130–134, figs. 73–75, see *Polyplastron;* for pp. 135–152, figs. 76–86, see *Ostracodinium;* for pp. 152–155, figs. 87–88, see *Enoploplastron;* for p. 156, fig. 89, see *Epidinium*).
>
> *Metadinium* Crawley, *partim*, 1923, p. 403, pl. 28, fig. C2; for pp. 403, pl. 28, fig C1, see *Metadinium;* for pp. 403, pl. 28, fig. C3, see *Ostracodinium*).
>
> *Anoplodinium*, Dogiel, *partim*, 1927, pp. 72–75, 77–105, figs. 40–56 (for pp. 75–77, figs. 37–39, see *Eodinium*).

Diagnosis.—Ophryoscolecidae with dorsal membranelle zone on the same level as the adoral zone; no skeletal plates present; macronucleus beneath right surface of the body; the anterior third of the dorsal surface of the macronucleus bent ventrally at an angle of 30°–90°; two contractile vacuoles present.

Type.—*Diplodinium dentatum* (Stein 1858) Schuberg 1888, from domestic cattle from Pavia, Italy.

This genus includes those species of Dogiel's subgenus *Anoplodinium* which possess the macronucleus considered as typical of *Anoplodinium* by Dogiel. The anterior third of the dorsal surface of this type of macronucleus bends ventrally at an angle of 30°–90°. It is always located under the middle of the right surface of the body. It was shown above that the shape and position of this type of nucleus are constant, even during the reorganization attendant upon vegetative division. Other features of morphology further characterize the genus as a whole as follows: the operculum is relatively large and prominent; caudal spines are common and in many cases possess a complex fibrillar system; the boundary layer is heavy and the rectum large and well developed, often showing a complex fibrillar structure. Furthermore, the species of *Diplodinium* range in size from 55 to 210μ, and average 100μ, while those of *Eodinium* range from 32 to 60μ, and average only 48μ. In all these features *Diplodinium* presents an advance over *Eodinium*.

DENTATUM GROUP—

The *Diplodinium dentatum* group is marked by the broad, truncated, posterior end of the body. This feature separates the group from the *D. anacanthum* group, in which the posterior end is tapering and conical. The spines found in the *D. dentatum* group are relatively long and heavy. *D. quinquecaudatum* Dogiel 1925, and *D. dentatum* (Stein) Schuberg, comprise this group.

Diplodinium dentatum (Stein 1858) Schuberg 1888

Plate 4, figure 2; figures A, B, 5–7

Entodinium dentatum Stein 1858, p. 70.

Diplodinium dentatum Schuberg, 1888, p. 404; Railliet 1890, pp. 318–319; Eberlein 1895, 261–262.

Diplodinium denticulatum Fiorentini 1889, p. 15, pl. 2, fig. 4–5; Becker and Talbott, 1927, p. 353, fig. 16.

Diplodinium dentatum variety *denticulatum* Buisson 1923, pp. 122–123, fig. 44.

Diplodinium denticulatum forma *denticulatum* Dogiel 1925, pp. 611–612, fig. 1.

Anoplodinium denticulatum forma *denticulatum* Dogiel 1927, pp. 84–86, fig. 44.

non *Diplodinium dentatum* Fiorentini 1889, p. 14, pl. 2, fig. 3; Buisson 1923, pp. 120–121, fig. 43; Becker and Talbott 1927, pp. 353, 356, fig. 14.

Diagnosis.—Dorsal surface convex, ventral surface concave; six large, incurved caudal spines, ventral spine longest; dorsal spine a continuation of a heavy longitudinal dorsal rib which arises near the dorsal membranelle zone. Length 65–82μ, 10 specimens.

Description.—The body is relatively short and heavy (1.20–1.32 dorso-ventral diameters in length), sharply truncated at the anterior and posterior ends, and with a prominent longitudinal rib on the dorsal surface. The body is compressed laterally (0.72–0.84 dorso-ventral diameters). The oral area is of medium size (0.29–0.36 dorso-ventral diameters), inclined ventrally at an angle of about 20° to the main body axis, and to the left at an angle of about 15°. The dorsal membranelle zone is relatively short, but the inner lip is prominent and conceals the small dorsal disk. The outer lips of the two membranelle zones are continuous, being connected along the right and left sides of the operculum by slight, but distinct, ridges. From a side view the operculum is broad and heavy, and more prominent than in most species of *Diplodinium*.

The dorsal surface of the body is strongly convex, the ventral side is weakly concave. The two lateral surfaces are convex, the right side being more strongly arched.

Six heavy, incurved, caudal spines project from the periphery of the truncate posterior end. They are 0.17–0.35 dorso-ventral diameters in length. The ventral spine is the heaviest and longest. Two lateral spines arise on each side from low, longitudinal ribs which merge into the body surface anteriorly. The dorsal spine is a continuation of a heavy, prominent rib originating near the dorsal

membranelle zone. A deep, sharply marked cleft extends between the bases of the dorsal spine and of the right dorsal spine. This cleft narrows anteriorly and ends near the dorsal zone.

The macronucleus lies under the left surface with its dorsal edge lying along the deep lateral cleft. It is a heavy, rod-like body with the anterior third bent vertically at an angle of about 45°. This shape is constant but the length is variable, ranging from 25 to 50μ (0.48–0.90 dorso-ventral diameters in length).

The micronucleus is a spherical or slightly ellipsoidal body lying in a slight depression in the dorsal surface of the macronucleus, in the region where the macronucleus is bent ventrally. The micronucleus varies from 4 to 10μ in diameter.

The two contractile vacuoles lie in the dorsal rib slightly to the left of the mid-line. There is a small pore and short excretory canal from the surface to each vacuole. The anterior vacuole lies a short distance behind the dorsal zone, the posterior vacuole at the level of the posterior end of the macronucleus. This latter vacuole is small and is usually difficult to distinguish.

The gullet and oesophagus are marked by a small tubular bundle of fibrils. These fibrils extend posteriorly, spreading laterally and dorsally. They terminate near the middle of the macronucleus.

The endoplasmic sac starts abruptly near the anterior end of the macronucleus. It extends posteriorly and is abruptly truncated near the bases of the caudal spines. The ectoplasm along the sides and ventral surface of the sack is thin, but heavy on the dorsal surface. The boundary membrane is relatively thin and difficult to distinguish, even in stained preparations. The rectum is a short, thin-walled tube lying at the base of the ventral spine. It is compressed along the dorso-ventral axis. It extends dorsally from its origin at an angle of about 50° with the main axis. The anus is elliptical. The rectum and anus are seen with difficulty.

The food is composed of bacteria and small particles of cellulose.

Variations.—The only striking variation observed is in the length of the spines, these ranging from 0.17–0.35 dorso-ventral diameters in length. Secondary spines arising from the main caudal spines have been reported by Dogiel (1927), but no example of this was found by us in the Indian material.

Measurements.—The following table is a summary of measurements of 10 individuals picked at random, with the measurements given by Dogiel (1927) listed for comparison:

Axis	From *Bos indicus*		From *Bos taurus* (after Dogiel)	
	Microns	Proportional	Microns	Proportional
Length	71 (65–82)	1.27 (1.20–1.32)	62 (55–70)	
Transdiameter	44 (40–50)	0.79 (0.72–0.84)		
Dorso-Ventral diameter	56 (52–62)	1.00	52 (44–60)	1.2
Macronucleus	43 (28–50)	0.78 (0.48–0.90)		
Mouth	18 (15–20)	0.32 (0.29–0.36)		
Tail	14 (12–20)	0.25 (0.17–0.35)	20–30	

Occurrence.—*Diplodinium dentatum* occurred in large numbers in all the *Bos indicus* examined, except in one individual from Colombo, Ceylon. This species has been reported in domestic cattle by Stein (1858) in the Republic of Czechoslovakia; in Italy by Fiorentini (1889) ; in Germany by Schuberg (1895) ; in California by Sharp (1914) ; in Iowa by Becker and Talbott (1927) ; and in the Union of Socialist Soviet Republics by Dogiel (1927).

Relationships.—The sharply truncated posterior end of *Diplodinium dentatum* is one of the striking features of this species. *Diplodinium quinquecaudatum* Dogiel (1925) also has this form and should be included as a close relative of *D. dentatum*. The other species which Dogiel includes in the denticulatum group, *D. anacanthum*, *D. monacanthum*, *D. diacanthum*, *D. triacanthum*, *D. tetracanthum*, *D. pentacanthum*, and *D. anisacanthum*, are conspicuously narrowed at the posterior end and should not be included in the *A. denticulatum* group.

Diplodinium quinquecaudatum Dogiel 1925

Diplodinium denticulatum variety *quinquecaudatum* Dogiel 1925c, pp. 613–617, figs. 2–7.

Anoplodinium denticulatum forma *quinquespinosum* Dogiel 1927, pp. 86–89, figs. 45a, b, 46a, b.

Anoplodinium denticulatum forma *quinquespinosum* aberration *calcar* Dogiel 1927, p. 88, fig. 46.

Diagnosis.—Five relatively large, straight, caudal spines present, one ventral, one dorsal, two on the right side, and one on the left; occasionally small secondary spines rise from the caudal spines. Length 65μ $(57–73\mu)$; dorso-ventral axis 55μ $(47–65\mu)$.

Occurrence.—In domestic cattle and sheep from Siberia (Dogiel, 1927).

This species was first described by Dogiel (1925) as *Diplodinium denticulatum quinquecaudatum*. In his Monograph of the Ophryoscolecidae (1927) he changed the forma name to *quinquespinosum* with no explanation of the change. Since trinomials are subject to the same rules of nomenclature as binomials, we have restored the original name *quinquecaudatum*.

Dogiel describes as typical those individuals with large, simple caudal spines. He noted in a small percentage of cases small secondary spines, or "thorns," arising from the main caudal spines, in most cases the right dorsal spine. To this latter type he gives the quadrinomial *A. denticulatum quinquecaudatum calcar*. Since the occurrence of these secondary spines is irregular and corresponds to the variations in spines as shown in *Ophryoscolex caudatus*, we do not feel that it merits a definite taxonomic rank.

ANACANTHUM GROUP—

This large group is marked by a tapering of the posterior half of the body, giving it a somewhat conical aspect. The development of the spines presents a complete series, equivalent to the *Epidinium ecaudatum* series. The species included in this group are: *Diplodinium*

anacanthum Dogiel 1927, *D. monacanthum* Dogiel 1927, *D. diacanthum* Dogiel 1927, *D. triacanthum* Dogiel 1927, *D. tetracanthum* Dogiel 1927, *D. pentacanthum* Dogiel 1927, *D. anisacanthum* da Cunha 1914, and *D. psittaceum* Dogiel 1927.

Diplodinium anacanthum Dogiel 1927

Anoplodinium denticulatum forma *anacanthum* Dogiel 1927, pp. 79–80, fig. 40a.

Diagnosis.—No caudal spines present. Length 80μ (70–90μ); dorso-ventral axis 51μ (40–60μ).

Occurrence.—In domestic cattle from U.S.S.R. (Dogiel 1927).

Diplodinium monacanthum Dogiel 1927

Plate 4, figure 5; figure D, 1, 2

Anoplodinium denticulatum forma *monacanthum* Dogiel 1927, pp. 79–80, 91, 92, fig. 40b.

Diagnosis.—Body relatively short and heavy; operculum low and flattened; rectum a narrow slit; a small ventral spine. Length 60–124μ, 10 specimens.

Description.—The body is relatively short and heavy (1.52–1.82 dorso-ventral diameters) and the operculum projects anteriorly only a short distance. The oral area is of moderate diameter (0.28–0.47 dorso-ventral diameters) and the lips weakly developed. The dorsal zone is large and conspicuous and the inner dorsal lip well developed. The dorsal disk is large and projects above the inner dorsal lip. The surfaces of the body are convex with the greatest curvature in the posterior third, the least curvature in the anterior third. The anterior third of the ventral surface is often slightly concave. The posterior half of the body is tapered, not truncated as in *D. dentatum*. A small spine from 5 to 8μ in length, projects from the posterior end of the ventral surface.

The macronucleus (0.52–1.02 dorso-ventral diameters in length) varies from a short, stout body only two or three times as long as its greatest diameter, to one five to six times as long as its greatest diameter. It is located under the right lateral surface. The anterior third of the macronucleus slopes ventrally at an angle of about 45°. There is a distinct bend in elongate macronuclei, in the short nuclei only the dorsal surface is bent, producing a triangular macronucleus. The micronucleus lies in a small depression of the dorsal surface of the anterior third of the macronucleus. The micronucleus is a small ellipsoidal body from 3 to 8μ in diameter.

The two contractile vacuoles are conspicuous lentoidal vesicles under the dorsal mid-line of the body, closely pressed against the boundary membrane. A small canal and pore connects each vacuole to the surface. A vacuole is located at each end of the middle third of the body. In many cases the posterior vacuole is larger than the anterior one.

The oesophagus, very weakly marked by fibrils, extends into the endoplasm, and ends close to the macronucleus. The endoplasmic sack starts anteriorly at the level of the anterior end of the macronucleus and extends posteriorly, closely following the contour of the body.

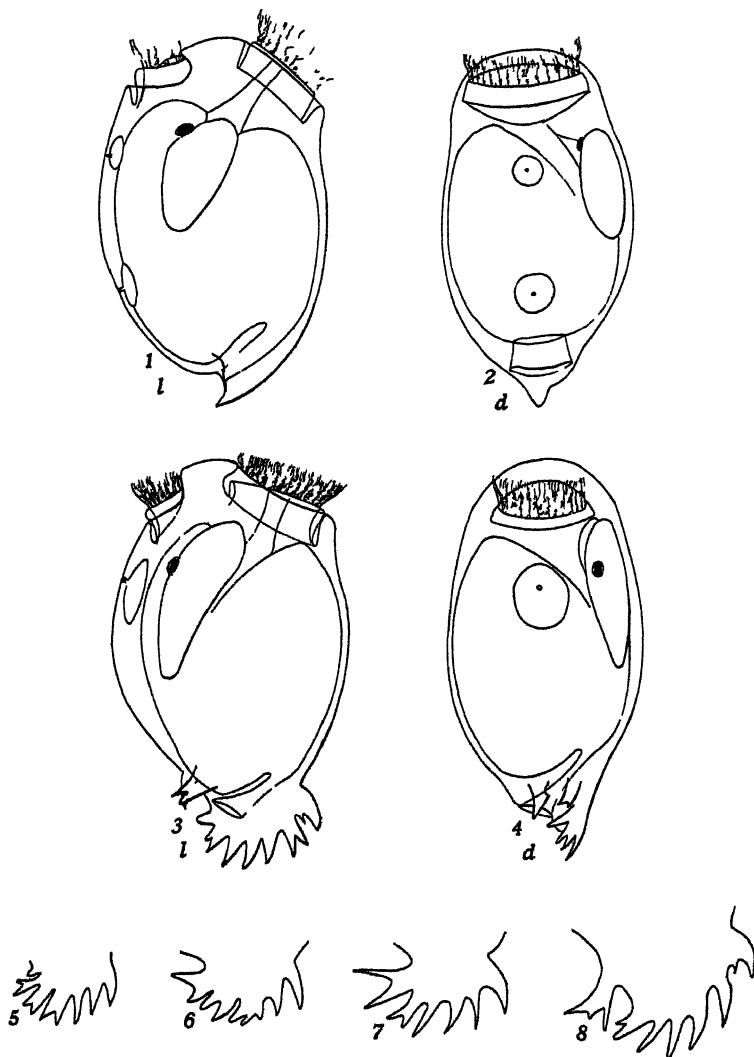Fig. D, 1 and 2, *Diplodinium monacanthum* Dogiel 1927; 3 and 4, *D. flabellum* sp. nov.; 5–8, variation in the caudal fan of *D. flabellum*. *l*, lateral view; *d*, dorsal view. × 500.

The rectum is a narrow slit with the walls extending a short distance anteriorly, parallel with the ventral surface. The anus is just dorsal to the ventral spine.

The food consists of small particles of plant material.

Variations.—There are no striking variations in this species.

Measurements.—The following measurements were taken from ten individuals picked at random. The measurements given by Dogiel are included separately.

Axis	From *Bos indicus*		From *Bos taurus* (Dogiel 1927)	
	Microns	Proportional	Microns	Proportional
Length	92 (60–124)	1.66 (1.52–1.82)	76 (60–88)	1.5
Transdiameter	49 (33–64)	0.90 (0.84–0 94)		
Dorso-ventral di-ameter .	55 (38–72)	1.00	55 (43–58)	
Macronucleus	44 (32–63)	0.82 (0.52–1.02)		
Mouth	20 (14–30)	0.36 (0.28–0.47)		
Tail	6 (5– 8)	0.10 (0.08–0.13)	5–10	

Occurrence.—*Diplodinium monacanthum* occurred in small numbers in one *Bos indicus* from Coonoor, India, and in all four from Colombo, Ceylon. Dogiel (1927) reports it in small numbers from cattle from U.S.S.R.

Relationships.—*Diplodinium monacanthum* belongs in the *Diplodinium anisacanthum* group because of its tapering posterior end, the proximity of the rectum to the ventral surface, and the smallness of the caudal spine.

Diplodinium diacanthum Dogiel 1927

Anoplodinium denticulatum forma *diacanthum* Dogiel 1927, pp. 80–81, fig. 41*a*, *b*.

Diagnosis.—Two caudal spines present, a ventral one of the same size as in *D. monacanthum*, and a dorsal spine shorter than the ventral spine. Length 75μ (70–83μ) ; dorso-ventral axis 52μ (47–60μ).

Occurrence.—In domestic cattle from U.S.S.R. (Dogiel 1927).

Diplodinium triacanthum Dogiel 1927

Anoplodinium denticulatum forma *triacanthum* Dogiel 1927, p. 81, fig. 42*a*, *b*.

Diagnosis.—Three caudal spines present: one ventral, one dorsal, and one on the right side. Length 77μ (70–85μ) ; dorso-ventral axis 55μ (51–64μ).

Occurrence.—In domestic cattle from U. S. S. R. (Dogiel 1927).

Diplodinium tetracanthum Dogiel 1927

Anoplodinium denticulatum forma *tetracanthum* Dogiel 1927, p. 82, fig. 2*c*.

Diagnosis.—Four caudal spines present: one ventral, one dorsal, and two on the right side. Length 76μ (72–83μ) ; dorso-ventral axis 54μ (52–61μ).

Occurrence.—In domestic cattle from U. S. S. R. (Dogiel 1927).

Diplodinium pentacanthum Dogiel 1927

Anoplodinium denticulatum forma *pentacanthum* Dogiel 1927, p. 82, fig. 42d.

Diagnosis.—Five caudal spines present: one dorsal, one ventral, and three lateral spines. Length 77μ (67–84μ); dorso-ventral axis 55μ (51–60μ).

Occurrence.—In domestic cattle from U. S. S. R. (Dogiel 1927).

Dogiel states that the lateral spines are usually arranged with two on the left side and one on the right, but occasionally specimens are found with only 'one on the left and two on the right side. This variation in position of the spines is unusual in the Ophryoscolecidae and may indicate that two different species have been placed together.

Diplodinium anisacanthum da Cunha 1914

Diplodinium anisacanthum da Cunha 1914, p. 64, fig. 3; Buisson 1923,
p. 123, fig. 44; Becker and Talbott 1927, p. 356.
Anoplodinium denticulatum forma *anisacanthum* Dogiel 1927, p. 83, fig. 42e.
Metadinium anisacanthum Crawley, 1923, p. 401; Fantham 1926, p. 568.

Diagnosis.—Six caudal spines: one dorsal, one ventral, and two on each side. Length 82μ (77–85μ).

Occurrence.—In domestic cattle from Brazil (da Cunha 1914); from U. S. S. R., and Turkestan (Dogiel 1927).

Becker and Talbott (1927) question the validity of this species, and consider it as an aberrant form of *Diplodinium denticulatum.* They have overlooked the fact, however, that the posterior end of *D. anisacanthum* is narrow and conical, while the posterior end of *D. dentatum* is broad and abruptly truncated. This difference, rather than the relative length of spines, is the distinguishing morphological feature between these two species.

Diplodinium psittaceum Dogiel 1927

Plate 4, figure 1; figure C, 1, 2

Anoplodinium psittaceum, Dogiel 1927, pp. 93–94, fig. 48.

Diagnosis.—Heavy, rounded body; thin ventral spine; a narrow dorsal flange on posterior third of body. Length 95–150μ, 10 specimens.

Description.—The bulbous body (1.34–1.61 dorso-ventral diameters in length) gives an impression of massiveness. It is compressed laterally (0.75–0.92 dorso-ventral diameters). This is the largest specimen of *Diplodinium* which we have found in *Bos indicus.* The oral area is relatively small in diameter (0.24–0.35 dorso-ventral diameters). It is inclined ventrally at an angle of 25°–30° and to the left at an angle of 10°–25°. The adoral region is large, with lips of moderate size. The operculum is short but is relatively broad and conspicuous. The dorsal disk also is large and conspicuous. The curvatures of the surfaces of the body in the anterior half are slight,

the greatest curvatures occurring in the middle and posterior parts of the body. The greatest curvature occurs in the posterior part of the dorsal surface. These marked curvatures give the posterior end a distinct subhemispherical shape. A low, narrow rib arises on the posterior half of the ventral mid-line and ends at the anus in a short, acute spine, from 6 to 15µ in length. A flange arises in the posterior quarter of the dorsal mid-line and disappears near the anus.

The macronucleus is a stout, rod-like body (0.56–0.82 dorso-ventral diameters in length), lying under the middle of the right surface of the body. It varies from three to six times as long as its largest diameter. Its anterior third is bent ventrally at an angle of approximately 40°. This bending is not so sharply marked as in most species of *Diplodinium*, but is nevertheless distinct.

The ellipsoidal nucleus, from 6 to 10µ in length, lies in a small depression on the dorsal surface of the anterior third of the macronucleus, with its long axis parallel to the long axis of the macronucleus.

The large lentoidal contractile vacuoles are located under the dorsal mid-line, lying close against the boundary layer. The anterior vacuole is located near the level of the macronucleus, the posterior vacuole is located in the posterior third of the body. A short canal and a small pore connect each vacuole with the surface.

The oesophagus is a long, loose, tubular bundle of fibrils terminating near the posterior end of the macronucleus. The endoplasmic sack arises close behind the membranelle zones and extends posteriorly, closely following the contours of the body. The boundary layer is distinct. The rectum is a short, dorso-ventrally flattened cylinder opening by an elliptical anus in the mid-region of the posterior end of the body. Dogiel (1927, fig. 48) figures heavy, circular myonemes surrounding the rectum.

Food.—The food consists of large pieces of plant material.

Variations.—The variations in this species are inconspicuous.

Measurements.—The measurements listed below were obtained from 10 specimens picked at random. The measurements given by Dogiel (1927) agreeing closely with ours, are placed in a separate column.

Axis	From *Bos indicus*		From cattle (Dogiel 1927)	
	Microns	Proportional	Microns	Proportional
Length	122 (95–150)	1.48 (1.34–1.61)	125 (105–155)	1.5
Transdiameter	70 (52– 90)	0.85 (0.75–0.92)		
Dorso-ventral diameter.	83 (60–105)	1.00	80 (59–100)	
Macronucleus	59 (40–85)	0.70 (0.56–0.82)		
Mouth	23 (20–30)	0.28 (0.24–0.35)		
Tail....	11 (6–15)	0.13 (0.09–0.16)		

Occurrence.—*Diplodinium psittaceum* occurred in fair numbers in all but one of the *Bos indicus* examined, this host was from Colombo, Ceylon. Dogiel (1927) found it once in Leningrad, U. S. S. R., in domestic cattle.

BUBALIDIS GROUP—

The *Diplodinium bubalidis* group is composed of those species which usually possess a small, longitudinal, cuticular groove extending a short distance anteriorly from the right border of the anus. The endoplasmic sack extends anteriorly into the operculum. There may be present a long, thin, ventral spine which narrows at the point of junction with the body, giving the effect of a movable joint. The species included in this group are: *D. bubalidis* (Dogiel 1925), *D. elongatum* Dogiel 1927, and *D. cameli* Dogiel (1926). *D. consors* (Dogiel 1925) is tentatively placed in this group.

Diplodinium bubalidis Dogiel 1925

Diplodinium bubalidis forma *bubalidis* Dogiel 1925b, pp. 124–140, fig. 3; 1925d, pp. 90–92, figs. 6, 7, 12; 1925a, pp. 287–412, figs. *F, N–P, H2–O2, M2, N2*; Fantham 1926, p. 567.
Anoplodinium bubalidis forma *bubalidis* Dogiel 1927, pp. 97–98, fig. 51.

Diagnosis.—Body oval with largest diameter anterior; ventral surface slightly convex, dorsal surface strongly convex; operculum prominent; a small, longitudinal groove on posterior part of right side of the body; the endoplasmic sack extends into the operculum; a single, thin, ventral spine present. Length 140μ ($104–195\mu$); dorso-ventral axis 80μ ($58–98\mu$).

Occurrence.—In antelope (*Bubalis cokei* and *Madoqua* sp.) from British East Africa (Dogiel 1925); domestic cattle from South Africa (Fantham 1926).

Diplodinium elongatum Dogiel 1927

Anoplodinium elongatum Dogiel 1927, pp. 95–97, fig. 50.

Diagnosis.—Body elongate (2.1 dorso-ventral diameters in length); dorsal and ventral surfaces weakly convex; operculum large; a narrow groove present in posterior end of right side of body; endoplasmic sack extends into the operculum. Length 195μ ($177–205\mu$); dorso-ventral axis 91μ ($73–100\mu$).

Occurrence.—In domestic cattle from U. S. S. R. (Dogiel 1927).

Diplodinium cameli Dogiel 1926

Diplodinium cameli Dogiel 1926, pp. 246–247, fig. 2.
Anoplodinium cameli Dogiel 1927, p. 94, fig. 49.

Diagnosis.—Body ellipsoidal and laterally compressed; operculum prominent; a small caudal lobe present; endoplasmic sack extends into operculum. Length 195μ ($160–210\mu$); dorso-ventral diameter 112μ ($92–130\mu$).

Occurrence.—In *Camelus dromedarius* from Turkestan (Dogiel 1926).

Diplodinium consors Dogiel 1925

Diplodinium bubalidis forma *consors* Dogiel 1925b, p. 124, fig. 3; Fantham 1926, p. 567.
Anoplodinium bubalidis forma *consors* Dogiel 1927, pp. 98–99, fig. 52.

Diagnosis.—Body oval; dorsal and ventral surfaces strongly convex; a long, thin, ventral spine present. Length 77μ (65–108μ); dorso-ventral axis 41μ (35–46μ).

Occurrence.—In antelope (*Bubalis cokei* and *Madoqua* sp.) from British East Africa (Dogiel 1927).

Dogiel (1927) states that there is no anterior projection of the endoplasmic sack into the operculum, and no longitudinal groove on the right side of the body in this species. This makes it questionable whether *D. consors* is nearly related to *D. bubalidis*. This species should be reinvestigated to determine its true relationship.

RANGIFERI GROUP—

A distinct, longitudinal, cuticular line running the length of the dorsal edge of the right lateral surface marks this group. The species included are relatively short (1.2–1.6 dorso-ventral diameters in length) and are roughly oval in lateral view. The ectoplasm is thick, and the rectum is relatively large and heavy. The species included within this group are *Diplodinium rangiferi* Dogiel 1925b, *D. minor* Dogiel 1925, *D. costatum* Dogiel 1925, and *D. dogieli* nom. nov.

Diplodinium rangiferi Dogiel 1925

Diplodinium rangiferi forma *major* Dogiel 1925c, pp. 49–51, fig. 9.
Anoplodinium rangiferi forma *major* Dogiel 1927, pp. 99–100, fig. 53.

Diagnosis.—Body broadly oval (1.22 dorso-ventral diameters in length); anterior end truncated, posterior end rounded; a narrow, longitudinal, cuticular thickening extends on the right dorsal surface from the anterior end of the body to the anus; endoplasmic sack does not extend into the operculum; rectum and anus large. Length 166μ (128–210μ); dorso-ventral diameter 136μ (110–165μ).

Occurrence.—In reindeer (*Rangifer tarandus*) from U. S. S. R. (Dogiel 1925).

Dogiel did not retain the specific name for any of the formas of *D. rangiferi*. We restrict the name *D. rangiferi* to the form described as *D. rangiferi major*.

Diplodinium dogieli nom. nov.

Diplodinium rangiferi forma *minor* Dogiel 1925*e*, pp. 51–52, fig. 10.
Anoplodinium rangiferi forma *minor* Dogiel 1927, p. 101, fig. 54.

Diagnosis.—Body broadly oval (1.29 dorso-ventral diameters in length); anterior end truncated, posterior end rounded; a narrow longitudinal thickening of the cuticle extends along the right dorsal surface from the anterior end of the body to the anus; the endoplasmic sack does not extend into the operculum; rectum and anus well developed. Length 89μ (70–100μ); dorso-ventral diameter 69μ (48–77μ).

Occurrence.—In reindeer (*Rangifer tarandus*) from U.S.S.R. (Dogiel 1925*d*).

Dogiel (1925*b*) described *D. costatum minor* and later (1925*d*) described *D. rangiferi minor*. In accordance with the rules of nomenclature we have substituted the name *Anoplodinium dogieli* for the name *A. rangiferi minor*, which was preoccupied.

Diplodinium costatum Dogiel 1925

Diplodinium costatum forma *major* Dogiel 1925*b*, p. 121, fig. 2.
Anoplodinium costatum forma *major* Dogiel 1927, pp. 102–103, fig. 55.

Diagnosis.—Body broadly oval (1.4 dorso-ventral diameters in length); truncated anteriorly, triangular posteriorly; a narrow longitudinal thickening of the cuticle extends along the right dorsal surface from the anterior end to the anus; rectum and anus small; endoplasmic sack extends into the operculum. Length 115μ (80–180μ); dorso-ventral diameter 81μ (55–110μ).

Occurrence.—In antelope (*Rhaphiceros* sp.) from British East Africa (Dogiel 1927).

Dogiel did not retain the specific name for any of the formas of *D. costatum*. We restrict the name *D. costatum* to the form described as *D. costatum major*.

Diplodinium minor Dogiel 1925

Diplodinium costatum forma *minor* Dogiel 1925*b*, p. 121, fig. 2.
Anoplodinium costatum forma *minor* Dogiel 1927, pp. 103–104, fig. 56.

Diagnosis.—Body oval (1.6 dorso-ventral diameters in length), truncated anteriorly, triangular posteriorly; a narrow longitudinal thickening of the cuticle extending along the right dorsal surface from the anterior end to the anus; rectum and anus small; the endoplasmic sack does not extend into the operculum. Length 75μ (60–90μ); dorso-ventral diameter 47μ (45–51μ).

Occurrence.—In African antelope (*Rhaphiceros* sp.) and bushbuck (*Tragelaphus scriptus*) from British East Africa (Dogiel 1925).

CRISTA-GALLI GROUP—

The *Diplodinium crista-galli* group includes those species with a roughly triangular lateral outline, truncate anteriorly, and tapering posteriorly. The rectum is relatively long and is circular in cross-section. *D. laeve* Dogiel 1927, *D. crista-galli* Dogiel 1927, and *D. flabellum* sp. nov. are included in this group.

Diplodinium laeve Dogiel 1927

Anoplodinium crista-galli forma *laeve* Dogiel 1927, pp. 91–93, fig. 47b.

Diagnosis.—Body roughly triangular; no caudal projections except a small ventral lobe; rectum tubular. Length 89μ (77–100μ); dorso-ventral diameter 61μ (52–70μ).
Occurrence.—In goats from northern Persia (Dogiel 1927).

Diplodinium crista-galli Dogiel 1927

Diplodinium crista-galli Dogiel 1927, p. 9.
Anoplodinium crista-galli forma *crista-galli* Dogiel 1927, pp. 91–93, fig. 47a, e, f.

Diagnosis.—Body triangular in lateral view, the left side extends posteriorly forming a prominent fan with two to seven spines. Length 89μ (77–100μ); dorso-ventral diameter 61μ (52–70μ).
Occurrence.—In goats from northern Persia (Dogiel 1927).

Diplodinium flabellum sp. nov.

Plate 4, figure 6; figure D, 3–8

Diagnosis.—Body roughly triangular in lateral view; the right side extends posteriorly, forming a prominent fan with five to seven spines; two small spines arise on posterior dorsal surface. Length 82–118μ, 10 specimens.
Description.—The body is relatively short (1.29–1.56 dorso-ventral diameters) and laterally compressed (0.79–0.91 dorso-ventral diameters). The lateral surfaces are strikingly like spherical triangles, with the membranelle zones located between their bases and the apices continued in the caudal armature. The oral area is of moderate size (0.30–0.41 dorso-ventral diameters). It is inclined ventrally at an angle of about 30° and to the left at an angle of about 15°. The dorsal membranelle zone is short, with a heavy inner lip which hides the small dorsal disk. The operculum is relatively small. There are no grooves along the base of the operculum connecting the outer furrows of the two membranelle zones.

The body tapers rapidly from the mid-region to the rounded posterior end. The ectoplasm of the right side continues beyond the apex and flares to form a broad fan (0.21–0.33 dorso-ventral diameters in length) with five to seven teeth extending radially beyond

its posterior margin. The majority of these teeth are simple, but several may be bifurcate or even trifurcate (fig. D, 8). Very small points may be present between some of the main teeth. Two additional spines arise on the dorsal surface near the base of the fan, one on each side of the mid-line. The left spine is simple, the right bifurcate.

The macronucleus is a heavy, rod-like body (0.32–0.85 dorso-ventral diameters in length), lying under the right surface. The diameter of its anterior end is usually two or three times the diameter of the posterior end. The anterior end is slightly bent ventrally. The ellipsoidal micronucleus, from 3 to 6μ in diameter, lies in a small depression on the dorsal surface of the anterior third of the macronucleus.

Only one contractile vacuole was observed. It is located under the mid-dorsal surface near to the dorsal zone. It is relatively large and strongly flattened dorso-ventrally. A short canal arises near the middle of the vacuole and opens on the surface by a small excretory pore. The posterior vacuole in many species of *Diplodinium* is exceedingly hard to observe. Since Dogiel reports a posterior vacuole in *D. crista-galli*, a closely allied species, a second vacuole may be present in *D. flabellum*.

The oesophagus is short and tubular, ending near the macronucleus. The endoplasmic sack arises near the anterior end of the macronucleus and closely follows the outer contour of the body to the posterior end. The boundary layer is strongly developed. The rectum arises on the right ventral side, passes dorsally and to the left, at an angle of 40°–50°. It is a narrow, tubular organ, flaring at the posterior end to form the circular anus. The anus lies in the mid-line and to the left of the caudal fan.

The food consists of small particles of plant material.

Variations.—The only variations observed were in the number and furcation of spines in the fan (fig. D, 5–8). No great reduction in the fan, as reported by Dogiel (1927) for *D. crista-galli*, was observed.

Measurements.—These measurements were taken from 10 specimens picked at random. Unfortunately, only 2 could be found which were expanded.

Axis	Microns	Proportional
Length	99 (82–118)	1.44 (1.29–1.56)
Transdiameter	58 (52– 65)	0.84 (0.79–0.91)
Dorso-ventral diameter	69 (57– 82)	1.00
Macronucleus	44 (41– 72)	0.63 (0.32–0.85)
Mouth	23 (20– 26)	0.35 (0.30–0.41)
Tail	18 (12– 25)	0.25 (0.21–0.33)

Occurrence.—*Diplodinium flabellum* was found in small numbers in three *Bos indicus* from Coonoor, India.

Relationships.—*Diplodinium flabellum* is similar to *D. crista-galli* in the general shape of the body, the shape of the rectum, and in the unique caudal fan of spines. *D. flabellum* possesses two dorsal spines which are lacking in *D. crista-galli*. The fan of *D. flabellum* is located at the right of the anus, but in *D. crista-galli* it is located at the left of the anus. This interesting transfer in position of a highly complex structure has not been noted in any other Ophryoscolecidae.

Eremoplastron gen. nov.

Eudiplodinium, Dogiel, *partim*, 1927, pp. 104–119, figs. 57–66 (for pp. 119–122, figs. 67*a*, *b*, see *Eudiplodinium*; for pp. 123–124, fig. 68, see *Diploplastron*; for pp. 124–130, figs. 69–72, see *Metadinium*).

Diagnosis.—Ophryoscolecidae with two membranelle zones, an adoral zone, and a dorsal zone lying at the anterior end of the body; a single, narrow skeletal plate beneath the right surface; triangular or rod-like macronucleus, anterior end often bent ventrally; two contractile vacuoles.

Type species.—*Eremoplastron rostratum* (Fiorentini 1889) from domestic cattle, Pavia, Italy.

The species included within *Eremoplastron* were formerly a part of the subgenus *Eudiplodinium* Dogiel 1927, most of them being included as "formas" of *E. neglectum*. *Eremoplastron rostratum* is the oldest species in this genus and one of the best known of this group, so we have designated it as the type species of *Eremoplastron*.

CHARACTERS OF SYSTEMATIC IMPORTANCE

The single skeletal plate of *Eremoplastron* lies beneath the right surface. It is in the shape of a narrow triangle with the base lying close to the middle of the right side of the outer adoral lip. The plate extends posteriorly and dorsally to the middle of the body, at an angle of 30°–45° with the main axis of the body. It usually terminates in the midregion, but occasionally extends as far as the posterior end of the macronucleus.

The skeletal plate is made up of a meshwork of relatively rigid material enclosing small, irregular, prismatic spaces which contain material staining deeply with chlor-zinc-iodide and similar reagents. In material stained with this reagent, the plate is brought out clearly as a series of small, brown polygons separated by narrow, clear lines. The brown material is the material within the plate, the clear lines separating the polygons constitute the actual meshwork of the skeletal plate. The polygons, which are the outer ends of the skeletal prisms, are arranged in two, rarely more, longitudinal rows in the long, narrow posterior part of the plate. In the broad, anterior portion of the plate numerous additional prisms are inserted between the rows.

Eremoplastron is the simplest genus of the Ophryoscolecidae in which a skeletal plate occurs. This plate is the most important structure separating *Eremoplastron* from *Diplodinium*, in which the skeletal plate is lacking.

The macronucleus is a roughly triangular body, greatly elongated in a few species, but never extending more than two-thirds of the length of the body. It lies along the dorsal edge of the skeletal plate. The ventral side of the macronucleus is flat or concave, the dorsal surface is strongly convex. The anterior third of the macronucleus is often bent ventrally. The greatest dorso-ventral diameter of the macronucleus usually occurs in its anterior third. As a result, the usual lateral outline of the macronucleus is an oblique-angled triangle, and strikingly similar to that of the macronucleus of *Diplodinium*. In *Eremoplastron insigne* and *E. giganteum,* the macronu-
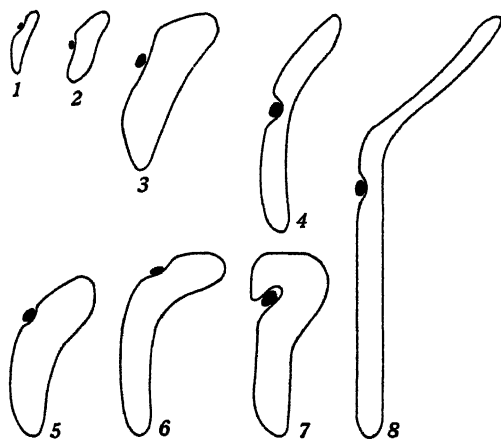

Fig. E. Types of nuclei, diagrammatic. 1, *Eremoplastron rostratum;* 2, *E. rotundum;* 3, *E. impalae;* 4, *E. insigne;* 5, *Diplodinium dentatum;* 6, *D. cameli;* 7, *Eudiplodinium maggii;* 8, *Eremoplastron giganteum.* 3, 4, 5, and 8 drawn after Dogiel (1927).

cleus is exceedingly elongate and slender, with the long side of the triangle toward the ventral surface, but the characteristic dorsal angle is retained, with the result that the macronucleus is a long, narrow bar with the anterior arm bent ventrally at an angle of 30°–40° with the posterior arm.

The micronucleus lies in a small, shallow depression in the dorsal surface. This depression is usually near the middle of the macronucleus, but in a few species it occurs nearer the anterior end.

The nuclear characters outlined above for *Eremoplastron* contrast strongly with those of *Eudiplodinium* and these two genera are most easily separated on this basis. In *Eudiplodinium* the anterior end of the macronucleus forms a large hook opening dorsally, with the

micronucleus lying within the hook and almost completely surrounded by the macronucleus. In *Eremoplastron,* because of the characteristic dorsal angle, the anterior arm of the macronucleus tends to bend ventrally, never dorsally as in *Eudiplodinium.*

Two contractile vacuoles occur in all species of *Eremoplastron.* They are located beneath the dorsal mid-line, one just posterior to the dorsal membranelle zone, and one at the level of the posterior end of the macronucleus. The walls of the vacuole are relatively thin and the excretory canals and pore are relatively small and inconspicuous. In *Eudiplodinium* the walls of the vacuole, the canal, and the pore are heavy and conspicuous.

Eremoplastron has a range in size unparalleled by any other genus of the Ophryoscolecidae. The species range from *E. rostatum,* which may be as small as 40μ, up to *E. giganteum* which is reported by Dogiel (1927) to reach a size of 500μ. Most of the species average about 80–90μ, but there is no range in size between that of the smallest and that of the largest species which is not occupied by that of at least one species.

The remaining morphological features such as proportions and shape of the body, shape and structure of the spines or lobes, size and proportions of the endoplasmic sack, and the shape of the rectum, exhibit the same morphology and types of variations described in *Diplodinium,* and therefore do not need to be discussed in full here. The above characters are of importance in specific classification and are taken up in detail in the descriptions of each species. The various species do not fall into closely bound groups as do the species of *Entodinium, Diplodinium,* and *Eodinium,* therefore no species groups have been indicated in *Eremoplastron.* Skeletal differences are not used because they are relatively small within the genus and the variations of size and shape of this structure between the individuals of a species are nearly as great as between the species. The difficulty of clearly locating the limits of the meshwork is of even greater importance from the standpoint of practical recognition of the species, and it is necessary to rely upon the appearance of the material within the meshwork after staining with one of the iodine reagents. The extent of the plate is not always fully revealed by this method, since the meshwork is often incompletely filled with this material.

Eremoplastron rostratum (Fiorentini 1889)

Plate 5, figure 7; figure F 1, 2

Diplodinium rostratum Fiorentini, 1889, pp. 16, 24, fig. 3; Eberlein, 1890, pp. 262–263, pl. 18, fig. 18; da Cunha, 1914b, pp. 62–64.
Eudiplodinium rostratum, Dogiel, 1927, pp. 118–119, fig. 66.
Diplodinium helseri, Becker and Talbott, 1927, 357–358, pl. 2, fig. 11.

Diagnosis.—Body proportionately long (1.58–2.00 dorso-ventral diameters in length) and compressed laterally; operculum projects anteriorly only a short distance beyond oral zone; thick dorsal flange and large ventral caudal spine; macronucleus rod-like; micronucleus lies in the middle of dorsal edge of the macronucleus; no projections of the endoplasm. Length 40–63μ, 10 specimens.

Description.—*Eremoplastron rostratum* is a small form, but relatively long (1.50–2.00 dorso-ventral diameters in length). It is compressed laterally to 0.80–0.88 dorso-ventral diameters. The dorsal surface is convex, the ventral surface nearly flat. The greatest dorso-ventral diameter is slightly anterior to the middle of the body. The oral region is relatively large (0.38–0.46 dorso-ventral diameters in diameter) and is tipped ventrally at an angle of about 20°, and to the left at an angle of about 10°–15°. The dorsal membranelle zone is small. The operculum, although relatively small, overhangs and partly obscures the dorsal membranelle zone.

A long caudal spine extends posteriorly from the region between the anus and the ventral surface. It ranges from 0.46–0.96 dorso-ventral diameters in length. The posterior third of the dorsal side of the body is thin and forms a flange-like projection.

The narrow skeletal plate lies under the right surface, extending from the edge of the oral zone to the middle of the body. The anterior end of the plate is four to five times as broad as the posterior end. The plate is composed of two to three rows of prisms in its posterior part, and four to six rows of prisms in the anterior part.

The macronucleus lies beneath the right surface of the body parallel to the skeletal plate and dorsal to it. It is an elongate body, 0.77–1.15 dorso-ventral diameters in length. The ventral side of the macronucleus is nearly straight, the dorsal surface is convex with a large median indentation in which the micronucleus lies. The greatest diameter of the macronucleus, both transverse and dorso-ventral, is in the portion anterior to this indentation. The micronucleus is a small ellipsoidal body from 2 to 4μ long.

The two contractile vacuoles lie beneath the dorsal surface along the mid-line. The anterior vacuole is located at the level of the micronucleus, the posterior vacuole lies in the anterior part of the dorsal flange. Both vacuoles open to the surface by short canals. The posterior vacuole is small and is often nearly invisible.

The mouth opens into the long, narrow oesophagus. The oesophagus extends posteriorly and to the right, terminating near the macronucleus and skeletal plate. The boundary layer is thin. The endoplasmic sack is oval and the ectoplasm is relatively thick. The rectum is a short, cylindrical structure extending dorsally from the posterior end of the endoplasmic sack at an angle of about 45°.
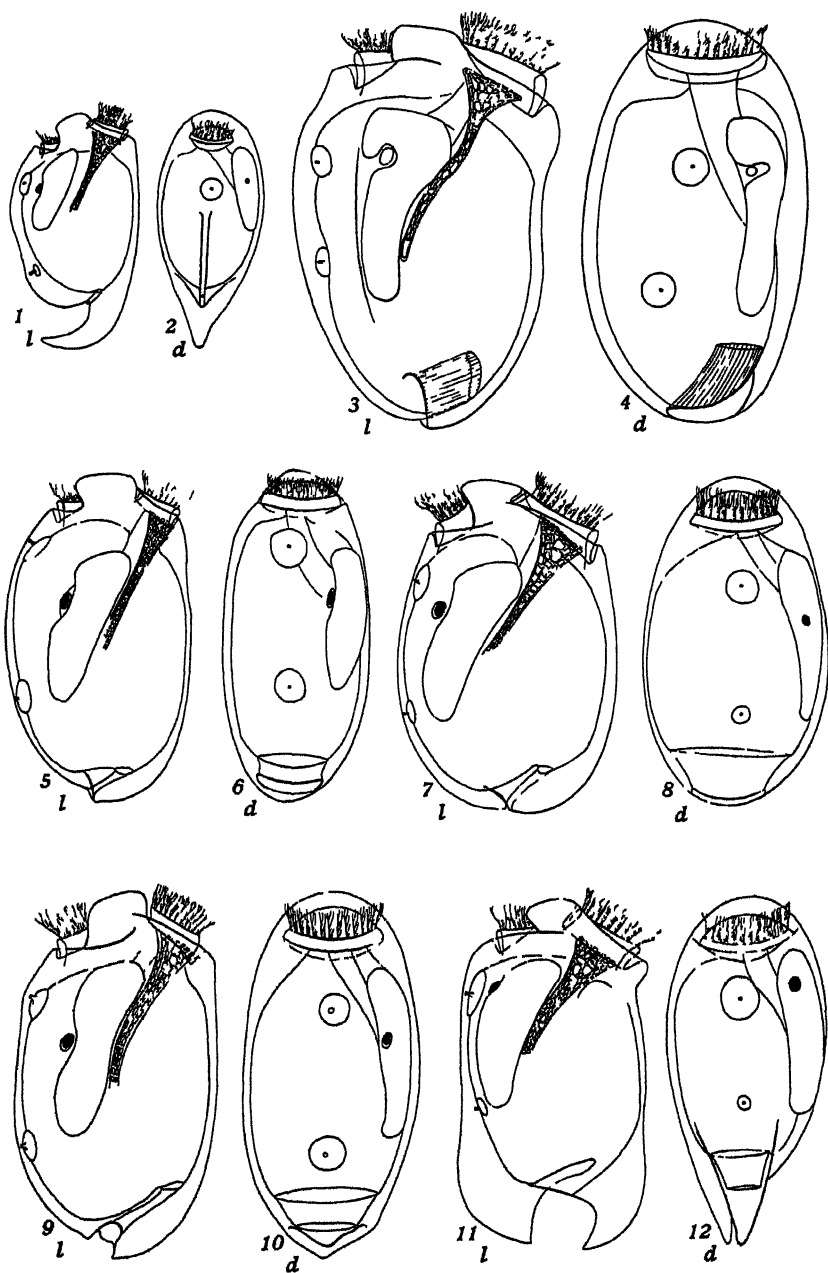
Fig. F. 1 and 2, *Eremoplastron rostratum* (Fiorentini 1889); 3 and 4, *Eudiplodinium maggii* (Fiorentini 1889); 5 and 6 *Eremoplastron bovis* (Dogiel 1927); 7 and 8, *Eremoplastron rotundum* sp. nov.; 9 and 10, *Eremoplastron brevispinum* sp. nov.; 11 and 12, *Eremoplastron magnodentatum* sp. nov., *l*, right lateral view; *d*, dorsal view. × 500.

Food.—Small bits of plant débris are found in the endoplasm.

Measurements.—The following measurements were made from 10 specimens picked at random from *Bos indicus.*

Axis	Microns	Proportional
Length of body	45 (40–52)	1.80 (1.58–2.00)
Transverse diameter	21 (19–23)	0.85 (0.80–0.88)
Dorso-ventral diameter	25 (22–26)	1.00
Macronucleus	24 (18–30)	0.93 (0.77–1.15)
Mouth	10 (9–12)	0.41 (0.38–0.46)
Caudal spine	15 (10–22)	0.60 (0.46–0.96)

Measurements given by other authors are listed below. Eberlein and Fiorentini have included the caudal spine as a part of the length, all the other authors have used only the length of the body.

	Fiorentini (1889)	Eberlein (1890)	Dogiel (1927)	Becker and Talbott (1927)
Length	80μ	70–80μ	54 (46–63)μ	43–58μ
Dorso-ventral diameter	40	40	34 (29–47)	25–33
Caudal spine			21 (15–29)	13–25

Occurrence.—*Eremoplastron rostratum* was reported from domestic cattle in Italy, by Fiorentini (1889) ; later it was found in Germany by Eberlein (1890) ; in Brazil by da Cunha (1914) ; in many parts of the U. S. S. R. by Dogiel (1927) ; and in Iowa by Becker and Talbott (1927). *E. rostratum* was present in small numbers in two of the *Bos indicus* examined, from Colombo, Ceylon.

Relationships.—The description and measurements of *Diplodinium helscri* Becker and Talbott 1927 fit *Eremoplatsron rostratum* closely, so we regard *D. helscri* as a synonym of *E. rostratum.*

E. rostratum is set off from the other species of the genus by its small size and the presence of the dorsal flange; only its generic characters unite it with the other species of this group.

Eremoplastron rotundum sp. nov.

Plate 5, figure 11; figure F, 7, 8

Diagnosis.—Body broadly ovoidal in side view (1.33–1.66 dorso-ventral diameters in length) with the largest diameter posterior; compressed laterally; operculum small; posterior end smoothly rounded; macronucleus rod-like; micronucleus lies in the middle of the dorsal edge of the macronucleus; postero-dorsal end of endoplasmic sack projects posteriorly beyond the rectum. Length 70–95μ, 10 specimens.

Description.—*E. rotundum* is relatively short (1.33–1.66 dorso-ventral diameters in length) and ovoidal in side view. The greatest dorso-ventral diameter is in the posterior half of the body. It is ellipsoidal in dorsal view.

The oral area is of medium size (0.28–0.36 dorso-ventral diameters in diameter), inclined ventrally at an angle of 30°–35°, and to the

left at an angle of about 15°. The dorsal zone is short, but the lips are well developed. The operculum is small and relatively inconspicuous.

The curvature of both dorsal and ventral surfaces is only slightly convex in the anterior half of the body, but increases rapidly in the posterior half so that the profile of the posterior end is nearly semicircular, with neither lobe nor spines to break the contour.

The narrow skeletal plate extends diagonally beneath the right surface from the edge of the oral region to the middle of the body. The narrow, posterior half of the skeletal plate is composed of two or three rows of rectangular prisms, but as the skeletal plate broadens anteriorly four or more rows of irregular prisms are added between the marginal rows.

The macronucleus lies in the middle half of the body adjacent to the dorsal edge of the skeletal plate. The ventral side of the macronucleus is flat or slightly concave, the dorsal side is strongly convex with a shallow median depression. In dorsal view the macronucleus is curved clavate with the blunt end anterior. Its curvature corresponds to the curvature of the right surface of the body.

The micronucleus is a small ovoidal body lying in the median depression of the macronucleus.

The two contractile vacuoles are located under the dorsal mid-line at about the levels of the ends of the macronucleus. They empty to the surface by short canals. The posterior vacuole is distinctly smaller than the anterior one.

The oesophagus is a narrow, tubular structure extending posteriorly from the mouth and to the left. It terminates near the end of the skeletal plate. The endoplasmic sack occupies the most of the body and follows the surface contours closely. The boundary layer is distinct. The ectoplasm is thin in most parts, but it is slightly thickened in the postero-ventral region. The rectum is wide and slit-like. It extends through the ectoplasm from the endoplasmic sack at an angle of about 45°. The anus is a narrow slit located in the middle of the posterior end.

Food.—The food consists of small bits of plant débris.

Measurements.—The following table is a summary of measurements of 10 specimens picked at random from *Bos indicus:*

Axis	Microns	Proportional
Length	86 (70–95)	1.48 (1.33–1.66)
Transverse diameter	50 (45–54)	0.86 (0.83–0.92)
Dorso-ventral diameters	58 (51–63)	1.00
Macronucleus	43 (40–50)	0.74 (0.60–0.98)
Mouth	19 (16–20)	0.33 (0.28–0.36)

Occurrence.—*E. rotundum* was found in all five of the *Bos indicus* from Coonoor, India, and in three of the hosts from Colombo, Ceylon.

E. rotundum is one of the simplest of the species of this genus. In its morphological characteristics it is closest to *E. neglectum, E. bovis,* and *E. spectabile,* all of which show a slight advance in complexity by the addition of a ventral lobe.

Eremoplastron neglectum (Dogiel 1925)

Diplodinium neglectum forma *neglectum* Dogiel 1925b, p. 127, fig. 4;
    Dogiel and Fedorowa, 1925, p. 99; Fantham, 1926, p. 567.
Eudiplodinium neglectum forma *neglectum,* Dogiel 1927, pp. 107–108,
    fig. 57.
Diplodinium neglectum Dogiel 1925e, pp. 54, 60; 1927, p. 20; Fantham
    1926, p. 567.

Diagnosis.—Body an elongate oval (1.78 dorso-ventral diameters in length); ventral surface slightly convex, dorsal surface strongly convex; operculum large; large ventral lobe; macronucleus long and rod-like; micronucleus lies on the middle of the dorsal side of the macronucleus; postero-dorsal end of the endoplasmic sack extends posteriorly beyond the anterior end of the rectum. Length, 81–124μ.

Occurrence.—*E. neglectum* was first reported from antelope (*Bubalis cokei* and *Madoqua* sp.) from British East Africa by Dogiel (1925), and later by Fantham (1926) from South African cattle.

Relationships.—*E. neglectum,* in structure and general shape, shows closest affinities to *E. bovis* and *E. brevispinum,* and differs from them mainly in proportions, the operculum in particular being larger than in the other two species.

Eremoplastron bovis (Dogiel 1927)

Plate 5, figure 10; figure F, 5, 6

Eudiplodinium neglectum bovis Dogiel, 1927, p. 108, fig. 58.
Anoplodinium neglectum forma *bovis,* Dogiel 1927, p. 244.
Diplodinium clevelandi Becker and Talbott 1927, pp. 356–357, pl. 2, fig. 20.

Diagnosis.—Body ellipsoidal (1.44–1.89 dorso-ventral diameters in length) and compressed laterally; operculum projects anteriorly only a short distance; a small caudal lobe present; macronucleus a rod-shaped body; micronucleus lies on the middle of the dorsal edge of the macronucleus; no projection of the endoplasmic sack. Length 52–100μ, 10 specimens.

Description.—The body in side view is a narrow ellipse (1.44–1.89 dorso-ventral diameters in length). The ventral surface is somewhat flattened except in the posterior quarter, where it curves dorsally to form the ventral lobe. The dorsal surface is more strongly convex than the ventral. The body is flattened laterally (0.78–0.95 dorso-ventral diameters on the transverse axis) and is elliptical in dorsal view.

The mouth is relatively small (0.19–0.33 dorso-ventral diameters in diameter). It is inclined ventrally at an angle of 25°–30° and to the left at an angle of 10°–20°. The dorsal membranelle zone is also small. The operculum is well developed and conspicuous although it projects anteriorly only a short distance.

A small, smoothly rounded, ventral lobe, from 1 to 3μ long, projects from the ventral half of the posterior end.

The skeletal plate is a narrow structure, lying in the ectoplasm beneath the right surface of the body. It extends diagonally under

the right surface from the edge of the oral region to the middle of the right side, where the individual prisms become smaller and disappear.

The macronucleus is an elongate body (0.64–1.03 dorso-ventral diameters in length). It lies beneath the middle of the right surface, with its ventral edge parallel and close to the skeletal plate. The ventral side of the macronucleus is straight, the dorsal side convex, with a conspicuous indentation in the middle. In dorsal view the macronucleus is seen to be curved in conformity with the curvature of the right surface.

The micronucleus is a small ovoidal body, from 3 to 5μ long. The nuclear membrane is separated from the chromatin mass by a very narrow clear space. The micronucleus lies adjacent to the left dorsal side of the macronucleus, in the dorsal indentation.

The anterior contractile vacuole is located a short distance posterior to the dorsal membranelle zone. The posterior vacuole is located at the level of the posterior end of the macronucleus. Both vacuoles lie beneath the dorsal surface along the dorsal mid-line. Each vacuole opens by a short excretory canal emptying on the surface through the small excretory pore.

The mouth opens into a narrow, tubular oesophagus, which extends posteriorly and toward the right surface. It ends in the endoplasm near the macronucleus. The fibrils lining the oesophagus are thin and inconspicuous.

The endoplasmic sack occupies the greater portion of the body. The ectoplasm is thin except at the anterior and posterior ends of the body, where it is greatly thickened. The boundary layer is easily seen, even in unstained material.

The rectum extends from the posterior end of the boundary layer at an angle of about 45°, and opens in the middle of the posterior end of the body at the base of the ventral lobe. The rectum is strongly compressed in the dorsal axis and is a narrow ellipsoid in cross-section. Faint fibrils lying parallel to the longitudinal axis are found in the wall of the rectum.

Food.—The food consists of small bits of plant débris.

Measurements.—The following measurements were made from 10 specimens from *Bos indicus.* The measurements given by Dogiel (1927) are also listed.

Axis	*Bos indicus*		Cattle and sheep (Dogiel 1927)	
	Microns	Proportional	Microns	Proportional
Length .	79 (52–100)	1.69 (1.44–1.89)	88 (78–100)	2
Transverse diameter ..	40 (34–50)	0.86 (0.78–0.95)		
Dorso-ventral diameter ...	47 (36–57)	1.00	44 (40–54)	
Macronucleus .	41 (23–52)	0.87 (0.64–1.03)		
Oral region	12 (10–19)	0.25 (0.19–0.33)		
Ventral lobe	2 (1– 3)	0.05 (0.02–0.07)		

Occurrence.—*Eremoplastron bovis* was found in three *Bos indicus* from Coonoor, India, and in three from Colombo, Ceylon. It was found in far greater numbers in the Ceylon material than in the Indian material. Dogiel (1927) reports it in cattle and sheep from various parts of the U. S. S. R. Becker and Talbott (1927) report it in cattle from Iowa.

Relationships.—*E. bovis* shows closest relationship to *E. neglectum,* which is relatively simple in structure and possesses a single ventral lobe. The operculum is a great deal smaller than in *E. neglectum.*

Eremoplastron brevispinum sp. nov.

Plate 5, figure 8; figure F, 9, 10

Diagnosis.—Body ellipsoidal (1.54–1.84 dorso-ventral diameters in length) and compressed laterally; operculum projects anteriorly relatively far; two short caudal spines; macronucleus rod-shaped; micronucleus lies on middle of dorsal surface of macronucleus; postero-dorsal end of endoplasmic sack extends into base of dorsal spine. Length 72–92μ, 10 specimens.

Description.—*Eremoplastron brevispinum* is ellipsoidal (1.54–1.84 dorso-ventral diameters in length) and compressed laterally (0.82–0.87 dorso-ventral diameters).

The mouth is small (0.16–0.29 dorso-ventral diameters in diameter). It is inclined ventrally at an angle of about 30°, and to the left at an angle of about 10°. The dorsal membranelle zone is relatively small. The operculum is conspicuous and extends anteriorly beyond the level of the oral zone. The right end of the outer dorsal lip continues two-thirds of the way across the base of the operculum, the left end of the outer adoral lip continues entirely across the base of the operculum and becomes continuous with the outer dorsal lip.

The dorsal surface is convex, with an even curvature throughout its entire length. The anterior half of the ventral surface is flat or slightly concave, the posterior half is convex. The lateral surfaces are convex and with the anterior and posterior surfaces form a nearly perfect ellipse in dorsal view.

Two short, broad spines, from 3 to 6μ in length, arise on the dorso-ventral mid-line. One spine lies just dorsal to the anus, the other spine lies ventral to the anus, and is merely a slight prolongation of a ventral lobe.

The skeletal plate is a narrow bar extending diagonally beneath the right surface from the edge of the oral zone to the middle of the body. The posterior three-quarters of the plate is made up of two or three rows of rectangular prisms, with their long axes parallel to the long axis of the plate. The anterior end of the plate is three to four times wider than the posterior part of the plate and is made up of four to six rows of plates.

The macronucleus lies beneath the right surface just dorsal to the skeletal plate. It is 0.49–0.96 dorso-ventral diameters in length. The ventral side of the macronucleus is flat or slightly concave, the dorsal surface is convex, with the greatest curvature in its anterior third. A large indentation occurs in the middle of the dorsal surface.

The micronucleus, a spherical or slightly ellipsoidal body from 3 to 6μ in diameter, lies in the indentation.

The two contractile vacuoles lie beneath the dorsal mid-line. They are relatively small. The anterior vacuole is found at the level of the anterior end of the macronucleus, the posterior vacuole lies just behind the level of the posterior end of the macronucleus. Each vacuole opens to the surface by a short and inconspicuous canal.

The oesophagus is a narrow tube leading from the mouth into the endoplasmic sack, extending dorsally and to the right, and terminating near the middle of the macronucleus.

The endoplasmic sack occupies the greater part of the body, the ectoplasm being very thin except at the anterior end and in the region of the rectum. The postero-dorsal part of the sack extends posteriorly beyond the anterior end of the rectum as in *E. magnodentatum* and *E. rotundum.*

The rectum is a wide, slit-like structure extending dorsally from the postero-ventral edge of the endoplasmic sack and at an angle of about 45°. Thin fibrils parallel to the long axis of the body lie in the wall of the rectum. The narrow, elliptical anus lies between the dorsal and ventral spines. The right edge of the anus is smooth, but the left edge is formed by a small, semicircular flap lying between the two spines.

Food.—The food consists of small bits of plant débris.

Measurements.—The following measurements were taken from 10 individuals picked at random from *Bos indicus:*

Axis	Microns	Proportional
Length	82 (72–92)	1.71 (1.54–1.84)
Transverse diameter	40 (37–44)	0.84 (0.82–0.87)
Dorso-ventral diameter	48 (42–53)	1.00
Macronucleus	37 (22–49)	0.90 (0.49–0.96)
Mouth	11 (7–13)	0.27 (0.16–0.29)
Caudal spines	4 (3– 6)	0.09 (0.06–0.11)

Occurrence.—*Eremoplastron brevispinum* was found in only one of the hosts examined, from Colombo, Ceylon.

Relationships.—The shape, proportions, and structure of *E. brevispinum* relate it closely to *E. bovis.* The development of the two spines of *E. brevispinum* shows a small advance in complexity over the single conical lobe of *E. bovis.*

Eremoplastron monolobum (Dogiel 1927)

Eudiplodinium neglectum forma *monolobum* Dogiel 1927, pp. 113-114, fig. 63a, b.

Diagnosis.—Body nearly spherical (1.3 dorso-ventral diameters in length) ; operculum small; prominent ventral lobe; a low, blunt dorsal lobe present; macronucleus thick and rod-shaped; micronucleus located in the middle of the dorsal edge of the macronucleus; no projections of the endoplasmic sack. Length 58–83μ.

Occurrence.—*E. monolobum* was reported by Dogiel (1927) from cattle from U. S. S. R.

Relationships.—E. monolobum is similar to *E. spectabile* and *E. impalae* in its structure and in the rotundity of the body. It is separated from the relatively large *E. spectabile* by its smaller size and from *E. impalae* by the median position of the micronucleus.

Eremoplastron spectabile (Dogiel 1925)

Diplodinium neglectum forma *spectabile* Dogiel 1925e, pp. 12, 59, 61, fig. 12.

Eudiplodinium neglectum forma *spectabile* Dogiel 1927, p. 109, fig. 59.

*Diagnosis.—*Body relatively short (1.46 dorso-ventral diameters in length); both dorsal and ventral surfaces strongly convex; operculum small; ventral lobe present; macronucleus stout and rod-shaped; micronucleus lies in the middle of the dorsal side of the macronucleus; postero-dorsal end of endoplasmic sack extends posteriorly beyond the anterior end of the rectum. Length 115–150μ.

Occurrence.—E. spectabile was reported by Dogiel (1925) from reindeer (*Rangifer tarandus*) from northern U. S. S. R.

Relationships.—E. spectabile shows close relationship to *E. impalae* and *E. monolobum* and differs from them mainly in its larger size.

Eremoplastron impalae (Dogiel 1925) emended

Diplodinium neglectum forma *impalae* Dogiel 1925b, p. 127, fig. 4; (for 1925e, pp. 52–53, see *P. tarandi*).

Eudiplodinium neglectum forma *impalae* Dogiel, *partim*, 1927, pp. 110–111, fig. 60b; (for pp. 110–111, fig. 60a, see *P. tarandi*).

*Diagnosis.—*Body relatively short (1.6 dorso-ventral diameters in length); operculum small; ventral lobe present; macronucleus with anterior end smaller than the middle; micronucleus located on the dorsal side of the anterior end of the macronucleus; postero-dorsal end of endoplasmic sack extends only a short distance beyond anterior end of rectum. Length 60–90μ.

Occurrence.—E. impalae was reported by Dogiel (1925) from the Impala antelope (*Aepyceros melampus*) from British East Africa.

Relationships.—E. impalae is closely related to *E. spectabile* and *E. monolobum* in its general structure and caudal armature, and especially in its shortness and rotundity of body.

Dogiel (1927) describes and figures a ciliate from *Rangifer tarandus* which he places in this species. The specimens from *Rangifer tarandus* are relatively shorter, a cuticular line is present near the dorsal edge of the macronucleus, and the anterior end of the macronucleus is relatively broad. The important morphological differences, and the wide difference in hosts and distribution make it impossible to include this ciliate from *Rangifer* with *E. impalae*, and accordingly we place it in a separate species, *E. tarandi*.

Eremoplastron tarandi sp. nov.

Eudiplodinium neglectum forma *impalae* Dogiel, *partim,* 1927, pp. 110–111, fig. 60a.

Diplodinium neglectum forma *impalae* Dogiel 1925e, pp. 52–53, fig. 11.

Diagnosis.—Body relatively short (1.36 dorso-ventral diameters in length) ; operculum small; a longitudinal cuticular line near dorsal edge of macronucleus; ventral lobe present; macronucleus narrowly clavate, with the blunt end anterior; the micronucleus located on the dorsal side of the anterior end of the macronucleus; postero-dorsal end of endoplasmic sack extends posteriorly only a short distance beyond anterior end of rectum. Length 74–105μ.

Occurrence.—*E. tarandi* was reported by Dogiel (1925e) from the reindeer (*Rangifer tarandus*) from northern U.S.S.R.

Relationships.—*E. tarandi* is closely related to *E. impalae* in its general structure and caudal armature, and especially in the shortness and rotundity of the body. It differs from *E. impalae* by the presence of a cuticular line near the dorsal edge of the macronucleus, and by the relative broadness of the anterior end of the macronucleus.

Eremoplastron dilobum (Dogiel 1927)

Eudiplodinium neglectum forma *dilobum* Dogiel 1927, pp. 116–117, fig. 65a, c.

Diagnosis.—Body ellipsoidal (1.5 dorso-ventral diameters in length), laterally compressed; operculum small; two caudal lobes, one dorsal and one ventral; macronucleus rod-shaped; micronucleus lies on middle of dorsal surface of the macronucleus; postero-dorsal end of endoplasmic sack extends into base of dorsal spine. Length 73–101μ.

Occurrence.—*E. dilobum* was reported by Dogiel (1927) from cattle and sheep from U. S. S. R.

Relationships.—The general shape and structure of *E. dilobum* give it an appearance quite similar to *E. magnodentatum,* the presence of caudal lobes instead of spines distinguishing it from the latter species.

Eremoplastron magnodentatum sp. nov.

Plate 5, figure 9; figure F, 11, 12

Diagnosis.—Body rectangular in side view (1.50–1.93 dorso-ventral diameters in length) ; compressed laterally; operculum small; two large, laterally compressed caudal spines, one ventral, one dorsal; macronucleus rod-shaped; micronucleus lies on the dorsal side of the anterior third of the macronucleus; postero-dorsal end of endoplasmic sack extends into the base of dorsal spine. Length 58–82μ, 10 specimens.

Description.—*Eremoplastron magnodentatum* is rectangular in side view (1.50–1.93 dorso-ventral diameters in length) and ovoidal in dorsal view, with the largest transdiameter anterior. The oral

region is 0.26–0.43 dorso-ventral diameters in diameter, and is inclined ventrally at an angle of 40°–50°, but is not inclined toward either side. The ventral slope is far more pronounced than in the other species of this genus. The outer adoral lip often projects ventrally beyond the ventral surface of the body. The adoral zone is well developed but the operculum is relatively small.

The dorsal surface is flat, the ventral surface is slightly convex. The dorsal and ventral sides in the posterior third of the body narrow and each forms a large, laterally flattened spine, 0.10–0.32 dorsoventral diameters in length. The outer edge of each spine turns sharply mediad while the inner edge of each spine is slightly oblique to the main axis of the body. Both spines are nearly equal in length, but the dorsal spine is thicker on the dorso-ventral axis than the ventral spine. The two caudal spines give a remarkable pincer-like appearance to the posterior end of the body.

The skeletal plate lies beneath the right surface and extends diagonally from the edge of the oral region toward the middle of the body. The posterior part of the plate is composed of from two to four rows of irregular prisms, and as the anterior third of the plate widens the prisms become larger and three or four extra rows are intercalated. The size of the skeletal plate is quite variable and in some individuals it is difficult to find, even when stained with chlor-zinc-iodide.

The macronucleus lies beneath the right surface close against the dorsal edge of the skeletal plate. The macronucleus is 0.74–1.00 dorsoventral diameters in length. The ventral surface is slightly concave, the dorsal surface is strongly convex, with a small, relatively deep depression in which the micronucleus lies. The micronucleus is an ellipsoidal body from 3 to 7μ in length. The size and position of the micronucleus is midway between the size and position of the large and small types of micronuclei found by Dogiel (1927) in two races of *E. bovis.*

The two contractile vacuoles lie beneath the dorsal mid-line, the anterior vacuole near the level of the micronucleus, the posterior vacuole near the level of the posterior end of the macronucleus. The excretory canals lead to the surface along the dorsal mid-line. The posterior vacuole is usually less than one-fourth the diameter of the anterior vacuole.

The endoplasmic sack extends from the bases of the membranelle zones to the posterior end of the body. In many species the endoplasmic sack extends only to the anterior end of the rectum. In *E. magnodentatum* a part of the endoplasmic sack bulges out into the base of the dorsal spine and extends beyond the rectum. This is even more noticeable in the description of *Eremoplastron bovis* given by Dogiel (1927).

The postero-ventral end of the endoplasmic sack opens into the slit-like rectum. The rectum extends posteriorly and dorsally at an angle of about 45° with the main axis. The elliptical anus opens in the base of the ventral spine.

Food.—The food consists of small bits of plant débris.

Measurements.—The following measurements were made from 10 specimens picked at random from *Bos indicus*:

Axis	Microns	Proportional
Length	70 (58–82)	1.68 (1.50–1.93)
Transdiameter	35 (26–44)	0.85 (0.79–0.87)
Dorso-ventral diameter	42 (30–50)	1.00
Macronucleus	37 (26–48)	0.88 (0.74–1.00)
Mouth	15 (12–20)	0.37 (0.26–0.43)
Caudal spines	10 (4–16)	0.23 (0.10–0.32)

Occurrence.—*Eremoplastron magnodentatum* was found in small numbers in one *Bos indicus* from Coonoor, India, and in one from Colombo, Ceylon.

Relationships.—*E. magnodentatum* shows closest relationship to *E. dilobum,* and in general shape they are strikingly similar. The development of the large, conspicuous caudal spines in *E. magnodentatum* may be considered as a slight advance over *E. dilobum* which has true caudal lobes.

Eremoplastron insigne (Dogiel 1925)

Diplodinium neglectum forma *insigne* Dogiel 1925, p. 127, fig. 4.
Eudiplodinium neglectum forma *insigne* Dogiel 1927, p. 111, fig. 61.

Diagnosis.—Body elongate (1.88 dorso-ventral diameters in length); operculum very large; no caudal spines nor lobes; small, cuticular fold in right surface of the body just dorsal to the rectum; macronucleus thin and rod-like; micronucleus lies in the middle of the dorsal surface of the macronucleus; anterior end of the endoplasmic sack projects posteriorly beyond the anterior end of the rectum. Length 124–222μ.

Occurrence.—*E. insigne* was reported by Dogiel (1925) in antelope (*Bubalis cokei* and *Madoqua* sp.) from British East Africa.

Relationships.—*E. insigne* and *E. giganteum* are similar in proportions, shape of the operculum, the anterior and posterior extensions of the endoplasmic sack, and the shape of the posterior end of the body. *E. insigne* forms a connecting link between the smaller members of the genus and *E. giganteum.*

Eremoplastron giganteum (Dogiel 1925)

Diplodinium neglectum forma *giganteum* Dogiel 1925b, p. 127, fig. 4.
Eudiplodinium neglectum forma *giganteum* Dogiel 1927, pp. 112–113, fig. 62.

Diagnosis.—Body elongate (1.9 dorso-ventral diameters in length); operculum large; no caudal spines or lobes; a deep cuticular fold on the right side of the body just dorsal to the rectum; macronucleus very narrow and rod-like, with the anterior third bent ventrally at an angle of about 45°; the micronucleus lies dorsal to and just behind the bend of the macronucleus; endoplasmic sack extends into the operculum; postero-dorsal end of endoplasmic sack extends posteriorly beyond the anterior end of the rectum. Length 256–500μ.

Occurrence.—*E. giganteum* was reported by Dogiel (1925b) from antelope (*Bubalis cokei* and *Madoqua* sp.) from British East Africa.

Relationships.—E. giganteum is very similar in general morphology to *E. insigne,* but is much larger and is the largest species of Ophryoscolecidae yet reported. *E. insigne* is midway in size between the ordinary range of size in the genus and the range of *E. giganteum,* so that there is a smooth gradation of size from *E. rostratum* (smallest size 40μ) to *E. giganteum* (largest size 500μ).

Eremoplastron rugosum (Dogiel 1927)

Eudiplodinium neglectum forma *rugosum* Dogiel 1927, pp. 114–116, fig. 64a–d.

*Diagnosis.—*Body short (1.5 dorso-ventral diameters in length), ventral surface flat or slightly concave, dorsal surface convex; a deep cuticular fold extends from the anus along the dorsal side of the macronucleus and terminates near the dorsal membranelle zone; ventral lobe compressed dorso-ventrally, with its dorsal border cut by from eight to ten shallow indentations; macronucleus long and rod-like; micronucleus lies on the anterior quarter of the dorsal surface of the macronucleus; postero-dorsal end of endoplasmic sack extends posteriorly beyond the anterior end of the rectum. Length 69–90μ.

Occurrence.—E. rugosum was found once by Dogiel (1927) in cattle in Leningrad, U. S. S. R.

*Relationships.—*The complex ventral lobe and the longitudinal groove along the dorsal side of the body in *E. rugosum* have no counterparts in the other species of the genus. The ventral lobe is unlike that of any other species of Ophryoscolecidae. The longitudinal groove, however, is very similar to the one found in *Diplodinium dentatum.*

Eudiplodinium Dogiel emended

Eudiplodinium Dogiel, *partim,* 1927, pp. 119–122, fig. 67a, b (for pp. 104–119, figs. 57–66, see *Eremoplastron;* for pp. 123–124, fig. 68, see *Diploplastron;* for pp. 124–130, figs. 69–72, see *Metadinium*).

*Diagnosis.—*Ophryoscolecidae with two membranelle zones, an adoral zone, and a dorsal zone lying at the anterior end of the body; a single, narrow, skeletal plate beneath the right surface; rod-like macronucleus with anterior end enlarged to form a hook opening dorsally; cuticle and ectoplasm thick; two contractile vacuoles with heavy membranes and prominent pores.

Type species.—Eudiplodinium maggii (Fiorentini 1889) Dogiel 1927, from domestic cattle from Pavia, Italy.

Eudiplodinium maggii is one of the most conspicuous and commonly noticed species of Dogiel's subgenus *Eudiplodinium.* For this reason, we have retained it as the type species of *Eudiplodinium* in the absence of any allocation of a type species by Dogiel. It is the only species belonging in this genus, with the possible exception of Fiorentini's *Diplodinium bursa.*

CHARACTERS OF SYSTEMATIC IMPORTANCE

The macronucleus of *Eudiplodinium* is the most conspicuous and characteristic structure. It lies beneath the right surface adjacent to the dorsal side of the skeletal plate. The anterior third of the macronucleus is greatly enlarged, with a very deep depression which opens dorsally, giving the shape of a very stout hook. The micronucleus lies in this depression. The posterior two-thirds of the macronucleus is a stout, heavy rod extending parallel to the main axis of the body. In small individuals the posterior part of the macronucleus is relatively short, while in large individuals it is relatively long. The anterior hook shows little difference in size in small and large individuals. During division, the characteristic hook appears in the posterior daughter long before the two daughters separate. The shape of the macronucleus of *Eudiplodinium* is entirely different from that of any of the other genera of Ophryoscolecidae, and there are no intermediate forms. Therefore, the shape of the macronucleus is considered to be the most important character defining *Eudiplodinium*.

The cuticle of *Eudiplodinium* is very heavy and resistant. The longitudinal striations on the surface are distinct. The ectoplasm, also, is thick and occupies a much larger proportion of the volume of the body than it does in *Eremoplastron*. The development of the cuticle and of the ectoplasm in *Eudiplodinium* is similar, though not so marked, as that in *Metadinium*.

There are two contractile vacuoles present, lying near the dorsal mid-line. The anterior vacuole is just behind the dorsal membranelle zone, the posterior vacuole is near the level of the posterior end of the macronucleus. The membranes of the vacuoles and the excretory canals are heavy, corresponding to the development of the cuticle. The excretory pore is large and often forms a conspicuous pit in the surface. The contractile vacuoles of *Eudiplodinium* contrast sharply with those of *Eremoplastron*, which have thin membranes and small, inconspicuous pores.

The remaining structures of *Eudiplodinium*, membranelle zones, skeletal plate, oesophagus, and rectum show no conspicuous differences from *Eremoplastron* and call for no further discussion.

Eudiplodinium maggii (Fiorentini 1889)

Plate 5, figure 12; figure F, 3, 4

Diplodinium maggii Fiorentini 1889, p. 13, pl. 1, figs. 3, 4; Eberlein 1895, pp. 252–256, figs. 8, 9; Buisson 1923, pp. 103–105, fig. 36; Dogiel and Fedorowa 1925, pp. 98, 100, 106, fig. 1; Becker and Talbott 1927, p. 353.

Diplodinium maggii Eberlein 1895, pp. 252–256, figs. 8, 9.

Diplodinium maggii Buisson 1923, p. 103.

Diplodinium bursa Schulze 1924, pp. 657, 661, fig. 5; Becker and Talbott 1927, p. 354, pl. 2, fig. 21.

Eudiplodinium maggii Dogiel 1927, pp. 119–122, fig. 67 a, b.

Diagnosis.—Body roughly triangular (1.37–1.67 dorso-ventral diameters in length); ectoplasm thick; anus opens on right side of posterior end; posterior end smoothly rounded; macronucleus with large dorsal hook on the anterior end; single skeletal plate. Length 104–255μ.

Description.—*Eudiplodinium maggii* is triangular in side view (1.33–1.67 dorso-ventral diameters in length), sharply truncated anteriorly and tapering to a blunt point posteriorly. It is flattened laterally to 0.86–0.97 dorso-ventral diameters, giving a rather narrow, elliptical outline in dorsal view.

The oral region is relatively small (0.20–0.33 dorso-ventral diameters in diameter) and inclined ventrally at an angle of 25°–35°, and to the left at an angle of about 10°. The dorsal membranelle zone is relatively large, while the operculum is relatively small and inconspicuous. The ends of the outer dorsal lip extend ventrally across the base of the operculum for some distance, occasionally as far as the outer adoral lip. The dorsal surface is convex. The anterior half of the ventral surface is flat or concave, while the posterior half is convex. There are no caudal projections.

A longitudinal cuticular fold is visible on the right surface just dorsal to the macronucleus. It fades out at the anterior end of the macronucleus, and may continue as far posteriorly as the edge of the anus. The right surface just dorsal to this line is depressed, often slightly below the level of the rest of the lateral surface.

The skeletal plate lies beneath the right surface and extends from the oral region dorsally across the middle of the body. The broad anterior end of the plate is composed of from eight to twelve rows of small prisms. As the plate narrows posteriorly the number of rows decreases and in the greater part of the length of the plate there are only two or three rows of prisms. The posterior end of the plate often terminates in a single, large prism.

The macronucleus is the most characteristic structure of *E. maggii*. It is an elongate, rod-like body, with the anterior end hooked dorsally. The micronucleus lies in the cavity of the hook. The shape is often described as "pistol-like." The macronucleus lies beneath the middle of the right surface, adjacent to the dorsal border of the skeletal plate. The micronucleus is an ovoidal body from 4 to 12μ in diameter. The chromatin is sometimes massed at one end, as Dogiel (1927) noted.

The contractile vacuoles lie beneath the dorsal surface near the mid-line. The anterior vocuole is at the level of the micronucleus, the posterior vacuole at the level of the posterior end of the macronucleus. Owing to the thickness of the ectoplasm, the excretory canals are relatively long and conspicuous. They extend toward the right dorsal surface and empty on the surface through large pores which often form pits in the surface. The anterior vacuole is often considerably larger than the posterior vacuoles.

The cuticle forms a distinct layer, prominent even in whole mounts. The ectoplasm is thick, *Metadinium medium* alone surpassing it in this respect.

The oesophagus is a long, tubular structure extending from the dorsal end to the right, terminating near the posterior end of the macronucleus. Fine longitudinal fibrils line the oesophagus.

The boundary layer is distinct and clearly marks out the endoplasmic sack.

The rectum is a heavy, slit-like organelle lying in the endoplasm at the posterior end of the body near the right surface. Heavy, longitudinal fibrils line the rectum. The anus also is displaced to the right, and is thus almost invisible from the left side.

Food.—The food consists of relatively large particles of plant material. The endoplasmic sack sometimes contains some of the smaller ciliates occurring in the same host. The large particles often badly distort the body.

Variations.—The cuticular line along the dorsal side of the macronucleus is highly variable. It may be entirely invisible, it may extend only a short distance, or it may extend from the anterior end of the macronucleus to the right end of the anus. The ventral edge of the anus is usually straight (fig. F, 3, 4), but may be slightly waved (pl. 5, fig. 12). The skeletal plate varies somewhat in length, but is usually conspicuous. The shape of the macronucleus is very stable and is modified only during the division stages.

Measurements.—The following measurements were taken from 10 individuals picked at random from *Bos indicus:*

Axis	Microns	Proportional
Length	140 (104–198)	1.55 (1.33–1.67)
Transdiameter	83 (56–120)	0.91 (0.86–0.97)
Dorso-ventral diameter	91 (63–125)	1.00
Macronucleus	73 (50–106)	0.80 (0.65–0.94)
Mouth	24 (16– 35)	0.27 (0.20–0.33)

The following measurements made by other authors are given to show the great range in size:

Author	Length	Dorso-ventral axis
Fiorentini (1889)	180	120
Eberlein (1895)	210 (190–240)	140 (130–170)
Dogiel (1927)	151 (115–212)	100 (73–143)
Becker and Talbott (1927)		
"D. maggii"	175–255	120–175
"D. bursa"	140 (100–150)	85 (60– 90)

Relationships.—There has been considerable confusion between *E. maggii* and *Diplodinium bursa* due to the somewhat incomplete descriptions of Fiorentini (1889). However, it is clear from his

drawings that there is a deep anal groove, or dorsal and ventral caudal lobes in his *D. bursa*, while his *D. maggii* has a rather pointed posterior end. Although the rectum and anus of *D. maggii* are conspicuous, there is no large anal groove. We consider that the ciliate described and figured by Becker and Talbott (1927) as *D. bursa* is in reality *Eudiplodinium maggii*, as it possesses all the characteristics of this species and is not bilobed posteriorly as described by Fiorentini in his *D. bursa;* and that the ciliate described by Becker and Talbott under the name of *D. maggii* is merely a large race of that species.

SPECIES INQUIRENDAE—

Diplodinium bursa Fiorentini 1889

Diplodinium bursa Fiorentini 1889, p. 14, pl. 2, figs. 1, 2; Buisson 1923*b*, p. 236; non Schulze 1924, pp. 657, 661, fig. 5; non Becker and Talbott 1927, p. 354, pl. 2, fig. 21.

Fiorentini described this species as similar to but smaller (100μ long and 60μ in dorso-ventral diameter) than *Eudiplodinium maggii*. The outstanding difference between the two species, both from his descriptions and from his drawings, is in the form of the posterior end. The posterior end of *E. maggii* is relatively sharp and with no lobes at all, while the posterior end of *Diplodinium bursa* possesses two large caudal lobes. Although recent authors have reported species under the name of *D. bursa*, these do not fit Fiorentini's description, and seem to be small races of *E. maggii*.

Diploplastron gen. nov.

Eudiplodinium Dogiel, *partim,* 1927, pp. 123–124, fig. 68 (for pp. 107–119, figs. 57–66, see *Eremoplastron;* for pp. 119–122, figs. 67*a, b,* see *Eudiplodinium;* for pp. 124–130, figs. 69–72, see *Metadinium*).

Diagnosis.—Ophryoscolecidae with dorsal and adoral membranelle zones at anterior end of body; two skeletal plates beneath the right surface; narrow, rod-like macronucleus; two contractile vacuoles below dorsal surface, separated from the macronucleus.

Type species.—Diploplastron affine (Dogiel and Fedorowa 1925) from cattle from U. S. S. R.

Although *Diploplastron* has two skeletal plates, it is more nearly related in structure to *Eremoplastron* than to *Metadinium*. It cannot be placed with *Eremoplastron* because of its two skeletal plates, and, on the other hand, because of the shape of the macronucleus, the thin cuticle and ectoplasm, and the small rectum and anus, it cannot be placed with *Metadinium*.

CHARACTERS OF SYSTEMATIC IMPORTANCE

The cuticle and ectoplasm of *Diploplastron* form only a thin layer, similar to that in *Eremoplastron neglectum* and its allied species. The complex fibrillar network present in the ectoplasm of *Metadinium* is absent in *Diploplastron.*

The skeletal plates lie beneath the right surface of the body, extending from the edge of the oral area past the middle of the body. The anterior ends of the plates are separated, while the posterior parts of the plates come close together, but do not fuse. Each plate is made up of from five to six rows of prisms. These plates are similar to those of *Metadinium,* and it is only on this basis that any close relationship can be traced between *Metadinium* and *Diplodinium.*

The rectum is a narrow, tubular structure with thin walls. The anus is small and circular. These structures of *Diploplastron* are similar to those in *Eremoplastron,* but are relatively much smaller than those in *Metadinium.*

This combination of structures gives *Diploplastron* a position between *Eremoplastron* and *Metadinium. Diploplastron* has the general structures of *Eremoplastron* combined with the two skeletal plates of *Metadinium* and may represent an evolutionary stage between *Eremoplastron* and *Metadinium.*

Diploplastron affine (Dogiel and Fedorowa 1925)

Fig. H, 2

Diplodinium affine Dogiel and Fedorowa 1925, p. 100.
Eudiplodinium affine Dogiel 1927, pp. 123–124, fig. 68.

Diagnosis.—Body small and oval (1.7 dorso-ventral diameters in length); operculum small; narrow rod-like macronucleus; endoplasmic sack extends posteriorly beyond anterior end of rectum; rectum narrow and tubular; anus circular.

Occurrence.—Dogiel (1927) reports *D. affine* from cattle, sheep and goats from various parts of U. S. S. R.
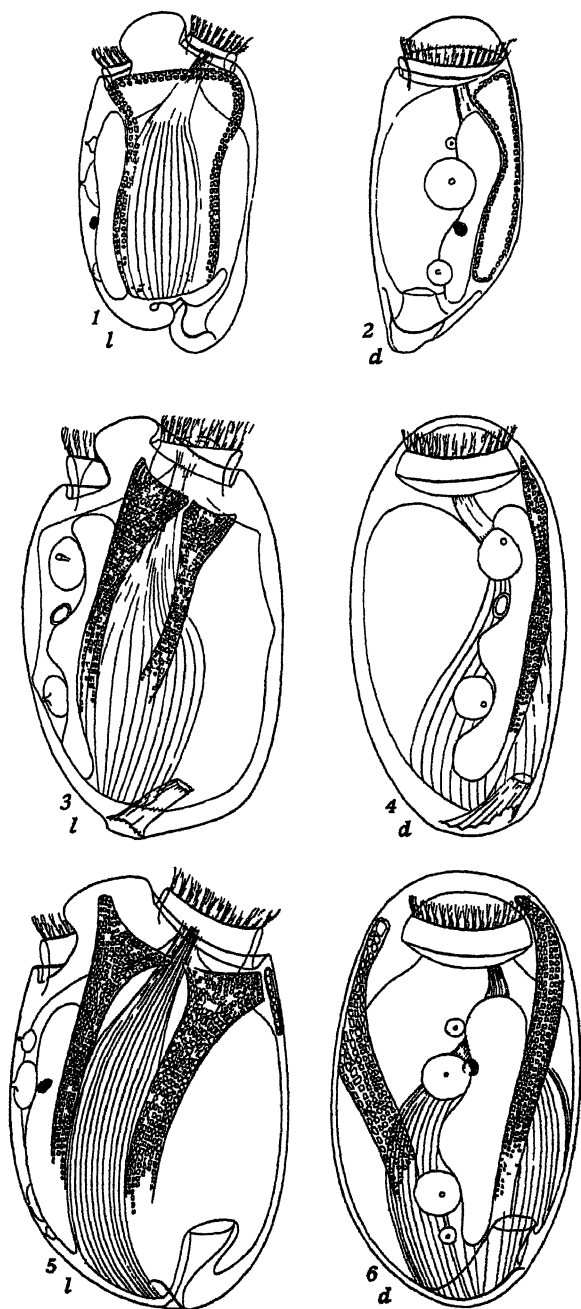
Fig. G.   1 and 2, *Ostracodinium mammosum* (Railliet 1890); 3 and 4, *Metadinium medium* Awerinzew and Mutafowa 1914; 5 and 6, *Elytroplastron bubali* (Dogiel 1928). *l*, right lateral view; *d*, dorsal view. × 500.
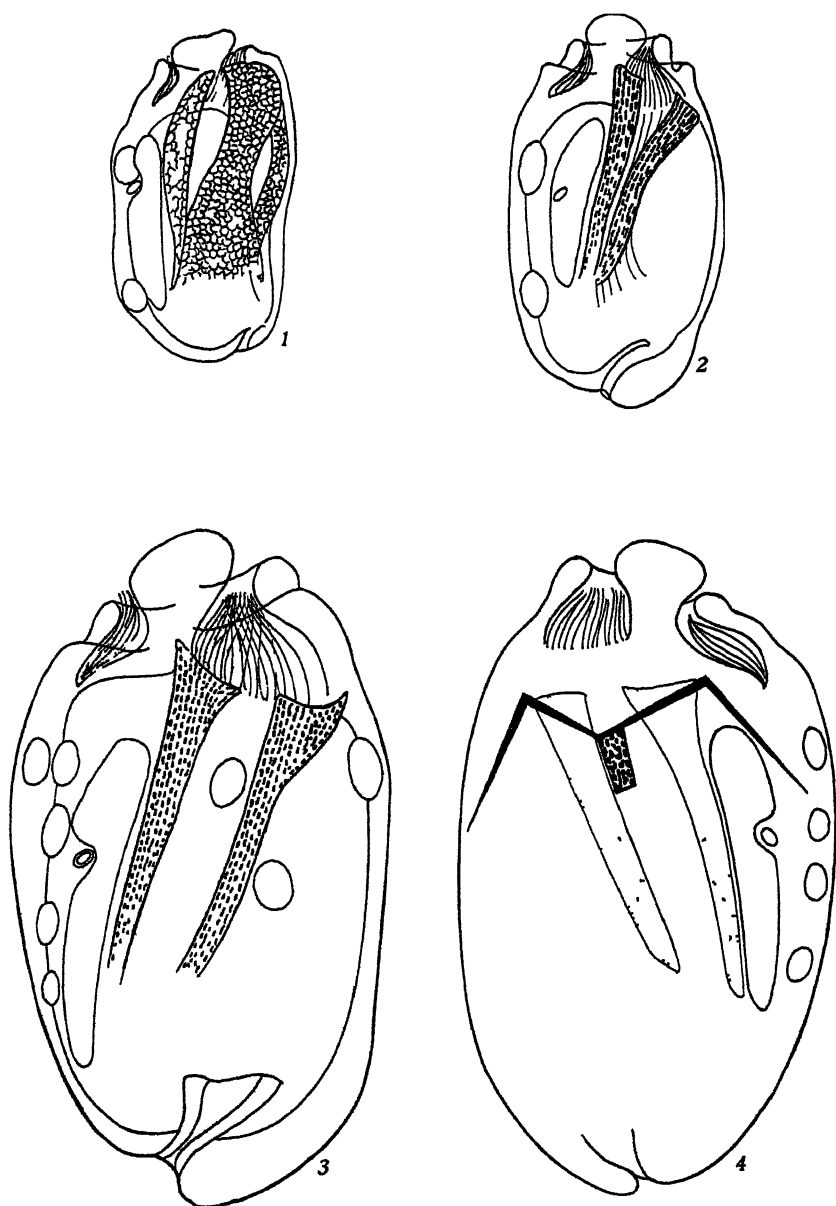
Fig. H. 1, *Enoploplastron triloricatum* (Dogiel 1925); 2, *Diploplastron affine* (Dogiel and Fedorowa 1925); 3, *Polyplastron multivesiculatum* (Dogiel and Fedorowa 1925), right lateral view; 4, left lateral view. Redrawn after Dogiel 1927.

<center>Metadinium Awerinzew and Mutafowa</center>

Metadinium Awerinzew and Mutafowa 1914, pp. 115–118, figs. 7–10; Crawley, *partim*, 1923, pp. 395, 400, pl. 28, fig. C1, (for p. 400, pl. 18, fig. C2, see *Diplodinium*, for p. 400, pl. 28, fig. C3, see *Ostracodinium*); non Fantham 1926, (for p. 568, see *Diplodinium*).

Eudiplodinium Dogiel, *partim*, 1927, pp. 124–130, figs. 69–72 (for pp. 107–119, figs. 57–66, see *Eremoplastron*, for pp. 119–122, figs. 67a, b, see *Eudiplodinium*, for pp. 123–124, fig. 68, see *Diploplastron*).

Diplodinium Becker and Talbott, *partim*, 1927, p. 354, fig. 24 (for p. 356, fig. 16, see *Anoplodinium*; for pp. 356–358, figs. 19–20, see *Eremoplastron*; for pp. 353–354, fig. 21, see *Eudiplodinium*; for pp. 356, 357, figs. 14, 17, see *Ostracodinium*; for pp. 354–355, figs. 22, 25, see *Epidinium*).

Diagnosis.—Ophryoscolecidae with dorsal and adoral membranelle zones at anterior end of the body; two skeletal plates beneath right surface, occasionally fused at posterior end; large macronucleus with two or three prominent dorsal lobes: two contractile vacuoles lying close to the macronucleus; cuticle and ectoplasm heavy; conspicuous oesophageal fibrils beneath dorsal and right lateral surfaces.

Type species.—*Metadinium medium* Awerinzew and Mutafowa 1914, from domestic cattle from U. S. S. R.

The original description of *Metadinium* emphasized the membranelle zones. Awerinzew and Mutafowa (1914) stated that the two membranelle zones of *Diplodinium* were connected on the left side, while in *Metadinium* this connection was lost. They also described the dorsal zone as a spiral instead of a semicircle as in *Diplodinium*. Buisson (1923, pp. 123–125), Dogiel (1927, pp. 124–126), and others, pointed out that the membranelle zones on *Diplodinium* are not connected, and that the dorsal zone of *Metadinium* is a semicircle as in *Diplodinium*, and so included *Metadinium medium* and other species of this group with Schuberg's *Diplodinium*. While *Metadinium* is not justified on the basis of the membranelle zones, it is separated from the other genera by its internal structures; skeletal plates, nuclei, contractile vacuoles, ectoplasm, etc., and it is necessary to retain *Metadinium* as a valid genus.

<center>CHARACTERS OF SYSTEMATIC IMPORTANCE</center>

The cuticle is very thick and heavy. There are no longitudinal striations on the surface of *Metadinium medium*, although there are often many fine, short wrinkles in the surface. The ectoplasm is very thick and in it are a large number of heavy, interlacing fibrils forming at feltwork near the cuticle (Dogiel 1927). In the other genera, longitudinal striations are present in the cuticle, but no interlacing mat of fibrils has yet been reported. Also, the thickness of the cuticle and ectoplasm is much greater in *Metadinium* than in other genera, including even *Euplodinium*.

The macronucleus is marked by two prominent dorsal lobes, one on the anterior end, one in the middle, and in *Metadinium medium* by a third one on the posterior end. Dogiel (1927) described these as a reversed F or E, respectively. The macronucleus lies beneath the right dorsal surface adjacent to the dorsal surface of the dorsal skeletal plate. The micronucleus is a small, ellipsoidal body lying anteriorly to the base of the middle lobe.

The macronucleus of *Metadinium* differs greatly from the simple, rod-like macronucleus of *Diploplastron,* one of the most nearly related genera. A macronucleus somewhat similar to that of *Metadinium* is found in *Ostracodinium gracile* and a few of its related species, but the dorsal lobes are not nearly so prominent as in *Metadinium.*

Two narrow skeletal plates occur beneath the right surface. The dorsal plate extends from the base of the operculum or from the edge of the dorsal half of the oral area, posteriorly across the middle of the body. The ventral plate extends from the edge of the ventral half of the oral area posteriorly to the middle of the body. In *Metadinium medium,* the commonest species, the plates are separate, but in *M. ypsilon, M. tauricum,* and *M. magnum,* the plates fuse posteriorly. The plates are composed of at least four longitudinal rows of prisms. The anterior end of each plate is somewhat wider than the rest of the plate and additional small prisms are intercalated between the rows in the anterior part.

The plates, in shape and position, are similar to those in *Diploplastron affine,* and to the plates under the right surface of *Polyplastron* and *Elytroplastron.* In *Polyplastron* the plates also are fused in a few species. The single plate found in *Eudiplodinium* and *Eremoplastron* is much narrower and composed of fewer rows of prisms than the plates of *Metadinium.*

The narrow, tubular gullet extends dorsally and to the right from the mouth through the anterior third of the endoplasm. In this region, the gullet expands and the fibrils lining the wall of the gullet separate to form the oesophagus. The oesophageal fibrils come close to the boundary layer on the dorsal and right side of the body and extend to the posterior end. These fibrils are relatively thick and heavy and are best seen in the whole mounts in glycerine. The oesophageal fibrils of *Metadinium* are homologous to the smaller and more irregularly arranged fibrils in such genera as *Entodinium, Diplodinium,* and *Eudiplodinium,* and are similar in form to the heavy fibrils in *Ostracodinium.* Since both *Metadinium* and *Ostra-*

codinium habitually ingest large pieces of plant débris, larger than those utilized by the other genera, the heavy oesophageal fibrils may serve as a protection to the more delicate body wall during ingestion of these particles.

Two contractile vacuoles lie to the right of the mid-line in the depressions between the dorsal lobes of the macronucleus. Their walls are heavy and the excretory canals are relatively long. The pores are conspicuous and, as in *Eudiplodinium,* lie in conspicuous pits. The pores open on the dorsal side of the right surface. The vacuoles are similar in structure to those of *Eudiplodinium.* In *Eudiplodinium,* however, the vacuoles are located near the mid-line, completely separated from the macronucleus, and in *Metadinium* they are at the right of the mid-line, in very close spatial relationship to the macronucleus.

The species of *Metadinium* are relatively large and heavy. The operculum is small in proportion to the size of the body. The membranelle zones are similar to those in *Eudiplodinium, Diploplastron,* etc. The rectum is large. The four species which have been reported, have no caudal projections. *Metadinium* shows no advance in any of these characters over the genera previously described, and in these characters shows closest similarity to *Eudiplodinium.*

Metadinium medium Awerinzew and Mutafowa 1914

Plate 6, figure 16; figure G, 3, 4

Metadinium medium Awerinzew and Mutafowa 1914, pp. 115–118, figs. 7–10.
Diplodinium medium Buisson 1923, pp. 123–124, fig. 45; Dogiel and
    Fedorowa 1925, pp. 100, 107, fig. 2; Becker and Talbott 1927, pp.
    353–354, fig. 24.
Eudiplodinium medium forma *medium* Dogiel 1927, pp. 124–126, fig. 69.

Diagnosis.—Large, heavy body (1.35–1.78 dorso-ventral diameters in length); oral and adoral membranelle zones large; operculum small; three dorsal lobes on the macronucleus; two narrow skeletal plates; large anus opens on right posterior surface. Length 180–272μ.

Description.—*Metadinium medium* is relatively broad and heavy (1.35–1.78 dorso-ventral diameters in length) and flattened laterally to 0.75–0.91 dorso-ventral diameters. The anterior end is blunt, the posterior end truncated or slightly rounded. The dorsal and ventral surfaces vary from nearly flat to distinctly convex. The lateral surfaces are slightly convex and the ends of the body in dorsal view are smoothly rounded.

The oral area is relatively large (0.27–0.41 dorso-ventral diameters in diameter). It is inclined ventrally at an angle of 20°–25°, but is not inclined to the left. The dorsal membranelle zone is also large,

with prominent lips. Awerinzew and Mutafowa (1914) described this zone as a spiral, similar to the oral spiral, but as Dogiel (1927), Becker and Talbott (1927), and others have shown, this zone is really a semicircle, as in the rest of the Ophryoscolecidae. The operculum is relatively very small. In the rest of the Ophryoscolescidae, the operculum is larger in proportion to the size of the species.

The two skeletal plates extend from the border of the oral area beneath the right surface toward the middle of the body. The anterior end of the dorsal plate extends from the base of the operculum across the dorsal half of the oral area; the anterior end of the ventral plate extends across the rest of the right side of the oral area. The anterior end of each plate is from two to three times the width of the posterior part. The dorsal plate is composed of about twelve to fifteen rows of small, rectanuglar or irregular prisms in its anterior part, and five to ten rows in it posterior part. The ventral plate is usually narrower than the dorsal plate and with two or three less rows than the dorsal plate. The dorsal plate extends beyond the middle of the body and is always longer than the ventral plate which usually terminates near the middle of the right surface.

The cuticle is very heavy and covered with short, fine wrinkles arranged longitudinally, but there are no regular striations such as occur in the other genera. The ectoplasm, also, is very thick.

The macronucleus is an elongate body lying adjacent to the dorsal edge of the dorsal skeletal plate. There are three large dorsal lobes, one at each end of the macronucleus and one in the middle, giving the macronucleus, from the left side, the appearance of an E. The macronucleus is 0.86–1.08 dorso-ventral diameters in length. The micronucleus is a small ovoid body lying in a slight depression along the anterior border of the middle lobe of the macronucleus.

The two large contractile vacuoles lie in the hollows between the lobes of the macronucleus. They lie somewhat to the right of the dorsal mid-line. The anterior vacuole is usually larger than the posterior vacuole. The excretory canals, leading to the surface, are relatively long, and the excretory pores are large and easily seen.

The mouth opens into a short, tubular gullet which gradually flares out and extends toward the right surface as the oesophagus. Heavy longitudinal fibrils line the gullet and oesophagus. In the posterior half of the body these fibrils are close against the ectoplasm of the right side and may be seen very easily in whole mounts cleared in glycerine. They appear as heavy, refractile bars extending from the middle of the body, toward the anus. These fibrils may be followed anteriorly from this line as they extend deep into the body and unite to form the tubular gullet. These heavy oesophageal bars probably correspond to the thin oesophageal fibrils found in such genera as *Entodinium*, *Eudiplodinium*, etc.

The endoplasmic sack is relatively small, due to the marked development of the endoplasm. The sack may be smoothly rounded, but it usually shows two distinct projections on the dorsal and ventral sides, giving the appearance of a bag supported at a few points instead of evenly over the whole surface. These projections are due to the structure of the ectoplasm and endoplasm, and are not due to

distortion from large food particles, since they may be seen in individuals with no such particles in the endoplasm.

The rectum is a large cylinder, slightly smaller at the anterior end than at the posterior end. It extends anteriorly into the endoplasmic sack beyond the general line of the ectoplasm. Heavy, longitudinal fibrils line the rectum. There is often a large vacuole of waste material adhering to the anterior end of the rectum. The anus is large, opening in the right posterior end of the body. The edge of the anus often appears slightly scalloped, due to the thickening of ectoplasm by the ends of the heavy rectal fibrils.

Food.—The food consists of relatively large bits of plant débris. These distort the body enormously, more so than in any other ciliate from ruminants.

Measurements.—The following measurements were made from 10 individuals selected at random from *Bos indicus:*

Axis	Microns	Proportional
Length	208 (180–224)	1.36 (1.25–1.78)
Dorso-ventral diameter	134 (111–143)	1.00
Transdiameter	110 (97–124)	0.83 (0.75–0.91)
Macronucleus	128 (107–155)	0.95 (0.86–1.08)
Mouth	44 (35– 50)	0.33 (0.27–0.41)

The following measurements are summarized from the literature:

Author	Length	Dorso-ventral diameter
Awerinzew and Mutafowa 1914	187–272	136–170
Dogiel 1927	186 (150–225)	140 (92–170)
Becker and Talbott 1927	187–270	136–175

Metadinium tauricum (Dogiel and Fedorowa 1925)

Diplodinium medium forma *tauricum* Dogiel and Fedorowa 1925), p. 100, fig. 3.

Eudiplodinium medium forma *tauricum* Dogiel 1927, pp. 126–127, fig. 70.

Diagnosis.—Large, heavy body (1.75 dorso-ventral diameters in length) ; operculum small; anterior and median lobes of macronucleus large, posterior lobe small; skeletal plates fused posteriorly; rectum and anus large. Length 185–288μ.

Occurrence.—Dogiel (1927) reports *Metadinium tauricum* in sheep, goats, and cattle from various parts of the U. S. S. R. and Persia.

Relationships.—*Metadinium tauricum* is nearest to *M. medium* in general structure. The chief differences from the latter species are in the partial fusion of the skeletal plates in *M. tauricum* and in the reduction of the posterior lobe of the macronucleus.

Metadinium ypsilon (Dogiel 1925)

Diplodinium ypsilon forma *ypsilon* Dogiel 1925*d*, p. 55, fig. 15.
Eudiplodinium ypsilon forma *ypsilon* Dogiel 1927, pp. 127–128, fig. 71.

Diagnosis.—Body oval (1.8 dorso-ventral diameters in length), laterally compressed, posterior end rounded; a small, anterior lobe and a median lobe on the macronucleus, no posterior lobe; skeletal plates fused posteriorly; rectum and anus small. Length 110–152μ.

Occurrence.—Dogiel (1927) reports *M. ypsilon* from cattle (two hosts only) from U. S. S. R.

Relationships.—*Metadinium ypsilon* is similar to *M. tauricum* in respect to the skeletal plates, macronucleus, and oesophageal fibrils, but is smaller, narrower, and more delicate in all respects than M. *tauricum.* The ectoplasm is thinner and the rectum is smaller than in *M. tauricum.* In all the above characters, *M. ypsilon* is similar to *M. magnum.*

Metadinium magnum (Dogiel 1925)

Diplodinium ypsilon forma *magnum* Dogiel 1925*e*, p. 55, fig. 14.
Eudiplodinium ypsilon forma *magnum* Dogiel 1927, pp. 129–130, fig. 72.

Diagnosis.—Body oval (1.64 dorso-ventral diameters in length), posterior end rounded; macronucleus with three, small, dorsal lobes; skeletal plates fused posteriorly; rectum and anus large. Length 156–201μ.

Occurrence.—Dogiel (1927) reports *M. magnum* from the reindeer (*Rangifer tarandus*) from northern U. S. S. R.

Relationships.—*M. magnum* is described by Dogiel as an enlarged form of *M. ypsilon.* The most outstanding differences between the two are size and proportions. The development of the oesophageal fibrils, according to Dogiel (1927), most nearly approaches the development in *Ostracodinium.*

Polyplastron Dogiel emended

Polyplastron Dogiel 1927, pp. 130–134, figs. 73–74; 1928, *partim,* p. 332
(for pp. 332–334, fig. 4*a, b,* see *Elytroplastron*).

Diagnosis.—Ophryoscolecidae with dorsal and adoral membranelle zones at anterior end of body; two skeletal plates beneath the right surface, either separate or fused together; three longitudinal plates beneath left surface with the anterior ends connected by transverse bars; a line of vacuoles beneath the dorsal surface, with additional vacuoles beneath the other surfaces.

Type species.—*Polyplastron multivesiculatum* (Dogiel and Fedorowa 1925) from domestic cattle from U. S. S. R.

CHARACTERS OF SYSTEMATIC IMPORTANCE

The five skeletal plates are the most striking structures in the genus and the most important taxonomic features. The two plates beneath the right surface are similar in position and form to those in *Diploplastron, Metadinium,* and *Elytroplastron.* Dogiel (1927) reports a few individuals in which the right plates were fused, either partly or completely, thus duplicating the fusion of the right plates in the species of *Metadinium.* The significance of the fusion from a taxonomic standpoint is treated under the discussion of *Polyplastron multivesculatum.* The skeletal complex on the left side of *Polyplastron* is composed of three plates connected by narrow bars. The plates are short, extending not more than a third of the length of the body. They extend from just behind the membranelle zones to the level of the micronucleus. Two of the plates are very narrow, one lying near the ventral side and one near the dorsal side. A short, broad plate lies midway between the narrow plates and slightly behind them. Two narrow connecting bars extend from the anterior end of the median plate, one going to the anterior end of each lateral plate, the whole complex having the shape of an M. Skeletal plates beneath the left surface are found in only one other genus, *Elytroplastron.* The complex of plates on the left side in *Polyplastron,* forming a single structure, is unique among the Ophryoscolecidae thus far reported and amply justifies generic distinction.

Four of the contractile vacuoles of *Polyplastron* are situated in the usual position, beneath the dorsal surface and near the macronucleus. Two vacuoles lie under the dorsal surface at the left of the anterior vacuole of the dorsal row. Two vacuoles lie near the middle of the right side, one between the skeletal plates, and one just below the ventral plate. Another vacuole lies under the ventral surface behind the oral region. In individuals with the right plates fused, the vacuole normally appearing between them is absent. The number of vacuoles approaches the condition in *Ophryoscolex;* but the vacuoles are not arranged as regularly as in the latter genus.

The remaining morphological structures present few features of value in the taxonomy of the genus. The ectoplasm is thicker than in most of the genera, but not nearly so thick as in *Metadinium.* The macronucleus is a straight rod-like body, slightly thicker in the anterior portion. A narrow, deep indentation in the middle of the

dorsal surface of the macronucleus receives the small micronucleus. The macronucleus lies adjacent to the right dorsal skeletal plate, as in all the genera with one or more skeletal plates. The rectum is large and thick-walled; the anus relatively small.

The original description of *Polyplastron* (Dogiel 1927) emphasizes the position and relationship of the skeletal plates and the position of the vacuoles. In a later paper (1928) Dogiel includes in *Polyplastron* a species, *P. bubali* from *Buffelus bubalus*. The skeletal plates of *P. bubali* differ greatly from those of *Polyplastron*. In particular, the M-shaped complex under the left surface of *Polyplastron* is replaced in this second species by a single long plate and there is present also a small ventral plate. We emphasize Dogiel's original description (1927) of *Polyplastron* and place his *P. bubali* in a new genus, *Elytroplastron.*

Polyplastron multivesiculatum (Dogiel and Fedorowa 1925)

Figure H, 3, 4

Diplodinium multivesiculatum Dogiel and Fedorowa 1925, p. 101, fig. 4.
Polyplastron multivesiculatum Dogiel 1927, pp. 130–134, figs. 73, 74.

Diagnosis.—Body oval (1.7 dorso-ventral diameters in length); operculum relatively large; row of four contractile vacuoles near the macronucleus, two vacuoles under the dorsal surface, one under the ventral surface, two under the right surface; two right skeletal plates separate; posterior end smoothly rounded. Length 120–190μ.

Occurrence.—*Polyplastron multivesiculatum* has been reported by Dogiel from cattle and sheep of U. S. S. R.

The two right skeletal plates are separate in most individuals of *P. multivesiculatum,* but in a few populations Dogiel found forms with the right skeletal plates either partly fused (*P. multivesiculatum* aberration *fenestratum*) or wholly fused to form a single plate (*P. multivesiculatum* aberration *confluens*). The question immediately arises as to whether these "aberrations" are merely individual variations or are forms with stable characteristics, i.e., separate species. In the other genera, the form of the plates is stable within a species and it seems unlikely that *Polyplastron* alone would be an exception. We separate the forms characterized by fusion of the plates from *P. multivesiculatum.*

Polyplastron fenestratum Dogiel 1927

Polyplastron multivesiculatum aberration *fenestratum* Dogiel 1927, pp. 133–134, fig. 75b.

Diagnosis.—Body oval (1.7 dorso-ventral diameters in length); operculum relatively large; row of four contractile vacuoles near the macronucleus, two vacuoles under the dorsal surface, one under the ventral surface, one under the right surface; right skeletal plates partly fused. Length (no measurements given).

Occurrence.—*Polyplastron fenestratum* has been reported by Dogiel from cattle from U. S. S. R.

Polyplastron monoscutum nom. nov.

Polyplastron multivesiculatum aberration *confluens* Dogiel 1927, pp. 133–134, fig. 75a.

Diagnosis.—Body oval (1.7 dorso-ventral diameters in length); operculum relatively large; row of four contractile vacuoles near the macronucleus; two vacuoles under the dorsal surface, one under the ventral surface; right skeletal plates fused into a single broad plate. Length (no measurements given).

Occurrence.—*Polyplastron monoscutum* has been reported by Dogiel from cattle from U. S. S. R.

The name *confluens* was used by Dogiel twice within *Diplodinium: Diplodinium (Polyplastron) multivesiculatum confluens* and *Diplodinium (Ostracodinium) triloricatum confluens.* We substitute *Polyplastron monoscutum* for the former species.

Elytroplastron gen. nov.

Polyplastron Dogiel, *partim*, 1928, pp. 332–334, figs. 4a, b (for p. 332, see *Polyplastron*).

Diagnosis.—Ophryoscolecidae with dorsal and adoral membranelle zones at the anterior end of body; two skeletal plates beneath right surface, a small plate beneath ventral surface, and a long plate beneath the left surface; cuticle and ectoplasm relatively heavy; conspicuous fibrils beneath dorsal and right lateral surfaces.

Type species.—*Elytroplastron bubali* (Dogiel 1928) from *Buffelus bubalus* from Georgia, U. S. S. R.

CHARACTERS OF SYSTEMATIC IMPORTANCE

Two skeletal plates of *Elytroplastron* are located beneath the right surface, one beneath the left surface, and a small plate beneath the ventral surface. The two plates beneath the right surface extend from the edge of the oral area posteriorly across the middle of the body. Each plate is composed of six or more rows of prisms. They are similar in form and position to the right lateral plates of *Diploplastron, Metadinium,* and *Polyplastron*. The plate beneath the left surface is a long, relatively narrow structure composed of four or five longitudinal rows of prisms. The plate is of the same width throughout, the anterior end does not flare out as in the right lateral plates, and the posterior end does not taper, but stops abruptly. The plate extends from the operculum diagonally across the left side, the posterior part extending beneath the dorsal surface and terminating near the posterior end of the macronucleus. The short, narrow ventral plate extends only a short distance posteriorly from the ventral edge of the oral zone.

The skeletal systems of *Elytroplastron* and *Polyplastron* show great differences. The single, long plate beneath the left surface of *Elytroplastron* is replaced in *Polyplastron* by three short plates forming an M-shaped structure. The ventral plate in *Elytroplastron* is entirely lacking in *Polyplastron*. The skeletal systems of the two are similar only in respect to the two right plates.

There is a row of four contractile vacuoles near the dorsal midline and near the macronucleus in *Elytroplastron bubali*. This is similar to the dorsal row of vacuoles in *Polyplastron*. The extra vacuoles found in *Polyplastron* are not found in *Elytroplastron bubali*.

The macronucleus of *Elytroplastron* is, in side view, an elongate body slightly curved, following the dorsal curve of the body. In dorsal view, two depressions in the left side of the macronucleus are sometimes seen in which the two middle contractile vacuoles lie.

The oesophageal fibrils of *Elytroplastron* are relatively large and arranged beneath the dorsal and right lateral surfaces as in *Metadinium*. Dogiel does not discuss the oesophageal fibrils of *Polyplastron,* so it cannot be compared in this respect to *Elytroplastron*. The cuticle and ectoplasm of *Elytroplastron* are somewhat heavier than in *Diploplastron*. The rectum and anus are relatively large.

The skeletal complex and oesophageal fibrils of *Elytroplastron* show an advance in complexity over *Diploplastron*. *Polyplastron*

exhibits approximately the same grade of complexity as *Elytroplastron*, although the paths of development of the two have been different. It may be suggested that *Elytroplastron* and *Polyplastron* have evolved from a form such as *Diploplastron* and in the same direction, i.e., toward the development of additional plates on the left side, but with the actual details of shape, number, and position of the plates differing.

Elytroplastron bubali (Dogiel 1928)

Plate 6, figures 13, 14; figure G, 5, 6

Diplodinium (Polyplastron) bubali Dogiel 1928, pp. 332–334, fig. 4.

Diagnosis.—Body ellipsoidal (1.43–1.82 dorso-ventral diameters in length) ; two right skeletal plates and left plate extend over half the length of the body, ventral plate very small; four contractile vacuoles along dorsal mid-line; posterior end smoothly rounded. Length 110–160μ, 10 specimens.

Description.—*Elytroplastron bubali* is an ellipsoid (1.43–1.82 dorso-ventral diameters in length), compressed laterally to 0 82—0.95 dorso-ventral diameters. The oral area is relatively large (0.36–0.51 dorso-ventral diameters in diameter). It is inclined ventrally at an angle of 25°–30°, but is not inclined either to the right or the left. The dorsal membranelle zone is relatively short. The operculum is broad but does not project anteriorly beyond the adoral zone. *E. bubali* is a nearly perfect ellipsoid, the smooth outline being broken only by the membranelle zones and the anus. Occasionally the ventral surface is slightly concave just behind the oral zone and just anterior to the anus. The posterior end is smoothly rounded, with no suggestion of either caudal lobes or spines.

Two skeletal plates lie beneath the right surface, similar to the plates of *Meladinium*. They extend diagonally from the edge of the adoral membranelle zone across the middle of the body, gradually fading out in that region. The anterior ends of the plates are over twice as wide as the posterior parts, and they become practically continuous at the anterior end, forming a semicircle around the right side of the adoral zone, extending from the operculum to the ventral side. There is one very short, triangular plate on the left ventral side with its anterior end just behind the adoral zone. A fourth plate lies beneath the left surface, extending posteriorly and dorsally from the base of the operculum. It terminates close to the posterior end of the macronucleus. The individual prisms of the plates are irregular polygons. A narrow, triangular area between the anterior ends of the ventral and left skeletal plates often stains deeply with chlor-zinc-iodide. It is not constant, however, and the granules of which it is composed are very irregular and smaller than the true skeletal prisms. This area, therefore, cannot be considered as a true skeletal plate.

The macronucleus is an elongate body (0.93–1.22 dorso-ventral diameters in length), lying slightly to the right of the mid-line. There is a deep indentation in the left dorsal side in which the small, ellip-

soidal micronucleus lies. In many individuals there are two large, shallow depressions in the left side of the macronucleus, one in the anterior third and one near the posterior end. There are four contractile vacuoles lying along the dorsal mid-line, one near the anterior end of the macronucleus, one at the level of the micronucleus, one at the posterior end, and one near the posterior tip of the macronucleus.

The narrow, tubular gullet extends posteriorly through the anterior third of the endoplasmic sack. The fibrils spread out in this region and become closely pressed against the boundary layer between the left dorsal side and the right side as far ventrally as the right ventral skeletal plate. The rectum is a heavy, tubular structure, in the postero-ventral end of the body. It lies beneath the right side of the body. The anus is a narrow slit extending from the middle of the posterior end to the right side. The position and shape of the rectum and anus are similar in both *Elytroplastron* and *Metadinium*.

Food.—The food consists of small pieces of plant débris and occasionally small ciliates.

Measurements.—The following measurements were taken from 10 individuals picked at random from *Bos indicus:*

Axis	Microns	Proportional
Length	132 (110–160)	1.69 (1.43–1.82)
Dorso-ventral diameter	77 (67– 97)	1.00
Transdiameter	67 (60– 78)	0.87 (0.82–0.95)
Macronucleus	83 (63–110)	1.08 (0.93–1.22)
Mouth	35 (28– 40)	0.45 (0.36–0.51)

Occurrence.—*Elytroplastron bubali* was first reported from *Buffelus bubalus* from Georgia, U. S. S. R., by Dogiel (1928). It occurred in two of the *Bos indicus* from Coonoor, India, and in two from Colombo, Ceylon.

Ostracodinium Dogiel emended

Ostracodinium Dogiel, *partim,* 1927, pp. 134–152, figs. 76–86 (for pp. 152–155, figs. 87–88, see *Elytroplastron*).

Diplodinium, Fiorentini, *partim,* 1889, p. 14, pl. 2, fig. 3 (for pp. 11–12, pl. 1, figs. 1–2, see *Ophryoscolex;* for pp. 13–14, pl. 1, figs. 1–4, see *Eudiplodinium;* for p. 15, pl. 2, figs. 4–5, see *Diplodinium;* for pp. 15–17, pl. 3, figs. 1–2, 4–5, see *Epidinium;* for p. 16, pl. 3, see *Eremoplastron*); Buisson, *partim,* 1923, pp. 120–121, figs. 35, 43 (for pp. 101–105, figs. 35, 36, see *Eudiplodinium;* for pp. 105–120, figs. 36–42, see *Epidinium;* for pp. 122–123, fig. 44, see *Diplodinium;* for pp. 123–125, fig. 45, see *Metadinium*); Fantham, *partim,* 1926, pp. 567, 568, fig. 7 (for p. 567, fig. 5, see *Eudiplodinium;* for p. 567, fig. 6, see *Eodinium;* for p. 567, see *Diplodinium;* for p. 567, 568, fig. 7, see *Ostracodinium;* for p. 567, see *Eremoplastron*); Becker and Talbott, *partim,* 1927, pp. 356, 357, figs. 14, 17 (for p. 354, fig. 24, see *Metadinium;* for p. 356, fig. 16, see *Diplodinium;* for pp. 356–358, figs. 19–20, see *Eremoplastron;* for pp. 353–354, fig. 21, see *Eudiplodinium;* for pp. 354–355, figs. 22–25, see *Epidinium*).

Metadinium, Crawley, *partim,* 1923, p. 400, pl. 28, fig. C3 (for pp. 395, 400, pl. 28, fig. C1, see *Metadinium;* for p. 400, pl. 18, fig. C2, see *Diplodinium*).

Diagnosis.—Ophryoscolecidae with dorsal and adoral membranelle zones at anterior end of body; broad skeletal plate beneath right side of body; a row of from two to six contractile vacuoles beneath dorsal surface; oesophageal fibrils heavy and extended to posterior end of body.

Type species.—*Ostracodinium mammosum* (Railliet 1890) from domestic cattle from Pavia, Italy.

Ostracodinium mammosum was the first of the species belonging to this genus to be described. It is easily identified by its peculiar caudal lobes, and is widely distributed. For these reasons and in the absence of a designation of a type species by Dogiel (1927), we designate *O. mammosum* as the type species of the genus.

CHARACTERS OF SYSTEMATIC IMPORTANCE

The single skeletal plate lies beneath the right surface. It extends laterally between the macronucleus and the ventral surface and longitudinally between the level of the membranelle zones and the level of the posterior end of the macronucleus. The ventral edge of the anterior end of the plate extends along the edge of the dorsal zone to the middle of the ventral side. The dorsal edge of the anterior
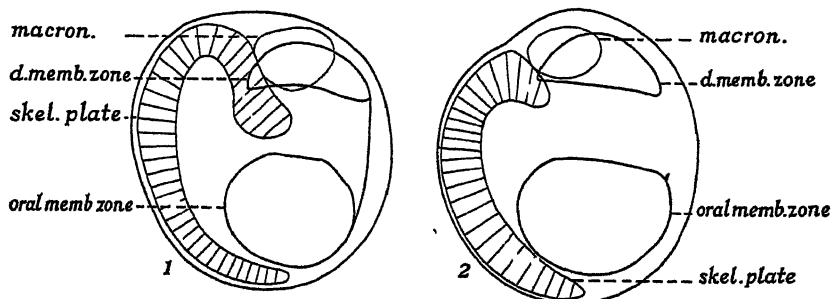
Fig. I. 1, *Ostracodinium rugoloricatum* sp. nov.; 2, *Ostracodinium gracile* (Dogiel 1925). Diagrammatic anterior views showing the relationships of the skeletal plate. *d. memb. zone*, dorsal membranelle zone; *macron.*, macronucleus; *oral memb. zone*, oral membranelle zone; *skel. plate*, skeletal plate.

end of the plate extends into the body a short distance (fig. I, 1). In most species of *Ostracodinium* the dorsal edge of the plate extends inward only a short distance, but in *O. rugosum* the plate extends a considerable distance inward (fig. I, 1). *Opisthotrichum* is the only other genus of the Ophryoscolecidae with a single skeletal plate of such great relative size as that in *Ostracodinium*. The finer structure of the plate of Ostracodinium is similar to that of the other genera. Many longitudinal rows of small prisms containing material staining

with the chlor-zinc-iodide reagent make up the plate. These prisms are shown in the plate figures, but in the text figures the individual prisms have been omitted in order to show the oesophageal fibrils more clearly, and only the outlines of the plates are drawn.

The contractile vacuoles are arranged in a longitudinal row near the dorsal mid-line. They lie close to the macronucleus, on its left dorsal side. They are thin-walled, with relatively small canals and pores leading to the surface. The number of vacuoles ranges from two to six, depending on the species. Dogiel (1927) considers that the number of vacuoles varies within a species. This point was the object of considerable attention from us. It was found that the contractile vacuoles are among the very first organelles to be duplicated in division, so that even when the only clear indication of division is the very small canal of the newly forming mebranelles of the posterior daughter, one or more extra vacuoles are already present. When these very early division stages are excluded, we have found that the number of contractile vacuoles is constant in a given species. Since all the species of *Ostracodinium* found in *Bos indicus* gave this result, we conclude that in cases where a varying number of vacuoles is reported, it is likely that either early division stages of the same species have been included inadvertently, or that two different species are being confused. The variation of the number of contractile vacuoles within the genus *Ostracodinium* is of interest since the number of vacuoles in most of the other genera, *Entodinium*, *Eudiplodinium*, *Diplodinium*, *Metadinium*, etc., is constant.

The oesophageal fibrils of *Ostracodinium* are highly developed, as in *Metadinium* and *Elytroplastron*. The narrow, tubular gullet extends dorsally and to the right from the mouth. In the anterior third of the endoplasmic sack the gullet expands and the fibrils thicken, forming the oesophageal fibrils. In this region they come close against the boundary layer and continue posteriorly to the end of the endoplasmic sack. Most of the fibrils lie beneath the right surface, but in some species there are fibrils beneath the dorsal surface as well.

The remaining features, particularly the operculum, macronucleus, rectum, anus, and caudal lobes, show many differences utilized in specific classification, but show no real advance over these features previously described in other genera. The macronucleus is relatively simple, but in a few species shows conspicuous indentations in which the vacuoles and micronucleus lie. The rectum is usually small and

varies in shape from a circular cross-section to a narrow elliptical one. There are usually fine longitudinal fibrils in its wall. Many of the species show no caudal lobes or spines. A few species show unusual shapes in the lobes (*O. monolobum* and *O. clipeolum*), and a large scoop-shaped lobe (*O. mammosum*). Five species possess spines. It is interesting to note that all of these have been reported only from the African antelope by Buisson (1923) and Dogiel (1927).

Ostracodinium mammosum (Railliet 1890)

Plate 7, figure 17; figure G, 1, 2

Diplodinium dentatum Fiorentini 1889, pp. 14, 24, pl. 2, fig. 3; non Eberlein 1895, pp. 261–262, fig. 39; da Cunha 1914*a*, p. 31; 1914*b*, 63, 64; 1917, pp. 2, 6; Sharp 1914, p. 60; Buisson 1923*a*, 120–121, fig. 35; Becker and Talbott, 1927, pp. 353, 356, pl. 2, fig. 14.

Diplodinium mammosum Railliet 1890, pp. 318–319; 1895, p. 181; da Cunha 1914*b*, p. 63.

Diplodinium fiorentinii Awerinzew and Mutafowa 1914, pp. 110–111, figs. 1, 2; Buisson 1923, p. 120, fig. 43.

Metadinium dentatum Crawley 1923, p. 400.

Ostracodinium dentatum Dogiel 1927, pp. 139–142, fig. 79*a*, *b*.

Diagnosis.—Body relatively short (1.55–1.92 dorso-ventral diameters in length); the posterior part of the skeleton extends only two-thirds of the way across the right side; macronucleus with large, shallow depression in middle of left side; three contractile vacuoles; one dorsal caudal lobe; a ventral lobe hollow on dorsal side. Length 41–110μ.

Description.—*Ostracodinium mammosum* is relatively short (1.55–1.92 dorso-ventral diameters in length). The oral area is 0.27–0.45 dorso-ventral diameters in diameter, and is inclined ventrally at an angle of about 25°, and to the left at an angle of 5°–10°. The adoral membranelle zone is relatively well developed. The operculum is large and extends anteriorly considerably beyond the oral area.

The ventral surface is convex in the anterior half, then becomes flat or slightly concave. In the posterior region, the curvature again becomes convex. The dorsal surface is convex throughout, with the greatest curvature in the posterior third of the body. The left surface is slightly convex. The anterior two-thirds of the right surface is only slightly convex, but the posterior third is strongly convex.

The dorsal surface terminates posteriorly in a narrow lobe extending ventrally to the middle of the posterior end. The ventral surface ends in a peculiar lobe-like structure in the shape of a scoop, from 8 to 15μ in length. The convex surface of the scoop is continuous with the ventral and lateral surfaces, the concave side opens dorsally. The dorsal ends of the scoop are longer than the ventral side, and in many individuals this is so prominent as to give the appearance of two separate, laterally flattened lobes.

The anterior end of the skeletal plate extends beneath the right surface from the anterior end of the macronucleus to the ventral side,

and posteriorly to the bases of the caudal lobes. The skeletal plate narrows posteriorly, so that only the extreme anterior end extends to the ventral side of the body.

The macronucleus is a long, rod-like body (1.13–1.40 dorso-ventral diameters in length), lying beneath the right dorsal surface. There is a large, shallow depression in the left side of the macronucleus. The median contractile vacuole lies near this region. The micronucleus is a small ellipsoid body, from 3 to 8μ in length, lying in a small depression near the middle of the dorsal surface of the macronucleus.

There are three contractile vacuoles present, lying beneath the dorsal surface of the body near the macronucleus. The anterior vacuole is found near the anterior end of the macronucleus, the middle vacuole just anterior to the micronucleus, and the posterior vacuole near the level of the posterior end of the macronucleus. The size of the contractile vacuoles in fixed material is much more variable than in other species of *Ostracodinium.*

The narrow, tubular gullet extends posteriorly and to the right from the mouth. In the anterior third of the endoplasmic sack it spreads out and comes close against the boundary layer beneath the skeletal plate. From this region to the posterior end of the endoplasmic sack, the heavy oesophageal fibrils can be seen clearly beneath the skeletal plate. The fibrils do not extend beneath the dorsal side.

The rectum is a dorso-ventrally flattened tube extending dorsally at a 45° angle from the postero-ventral end of the endoplasmic sack. The elliptical anus lies in the concave side of the ventral lobe.

Food.—The food consists of small bits of plant débris.

Measurements.—The following measurements were made from 10 individuals selected at random from *Bos indicus:*

Axis	Microns	Proportional
Length	82 (53–97)	1.74 (1.55–1.92)
Dorso-ventral diameter	47 (33–58)	1.00
Transdiameter	43 (30–54)	0.91 (0.86–0.93)
Macronucleus	56 (36–68)	1.20 (1.03–1.40)
Mouth	16 (10–20)	0.37 (0.27–0.45)
Caudal lobes	11 (8–15)	0.25 (0.17–0.39)

The following measurements are summarized from previous work on individuals from domestic cattle:

Author	Length in microns	Dorso-ventral diameter in microns
Fiorentini 1889	100	48
Awerinzew and Mutafowa 1914..	80– 92	44–60
Dogiel 1927	41–110	25–68
Becker and Talbott 1927	73–104	44–60

Occurrence.—*Ostracodinium mammosum* was first found in domestic cattle in Italy by Fiorentini (1889); later in various parts of the U. S. S. R. by Awerinzew and Mutafowa (1914) and Dogiel (1927); in Brazil by da Cunha (1914); and in the United States of America by Sharp (1914), and Becker and Talbott (1927).

Occurrence.—*O. mammosum* was fairly abundant in two of the *Bos indicus* from Coonoor, India, and in three from Colombo, Ceylon.

Relationships.—*Ostracodinium mammosum* resembles *O. dilobum* in the general form of the body and in the two caudal lobes. *O. mammosum* is smaller, and has fewer vacuoles, but the caudal lobes are relatively larger than in *O. dilobum.*

Ostracodinium gracile (Dogiel 1925)

Plate 7, figure 18; figure J, 1, 2

Diplodinium gracile forma *gracile* Dogiel 1925*b*, pp. 130, 133, 141, fig. 5;
1925*a*, pp. 297–401, figs. *B*, *E–J*, *K–L*, *E₁–M₁*, *E₂–G₂*.
Ostracodinium gracile forma *gracile* Dogiel 1927, pp. 144–146, fig. 81*d*.

Diagnosis.—Body roughly triangular; ventral and left surfaces plane, right and dorsal surfaces convex; anus at postero-ventral end of body; skeletal plate extends across right surface; macronucleus with two dorsal lobes; two contractile vacuoles; posterior end smoothly rounded. Length 90–133μ.

Description.—*Ostracodinium gracile* is roughly triangular with the membranelle zones forming the base of the triangle. The ventral zone is flat or slightly convex; the dorsal surface is strongly convex, with the greatest curvature in the posterior quarter. The left side of the body is flat or slightly convex, the right side is strongly convex. The posterior end is smoothly rounded.

The oral area is prominent (0.29–0.43 dorso-ventral diameters in diameter) and is inclined ventrally at an angle of 10°–20°, but is not inclined either to the right or the left. The dorsal membranelle zone is relatively prominent, as is the operculum.

The broad skeletal plate lies beneath the right surface and extends laterally from the macronucleus to the ventral surface.

The macronucleus is an elongate body (1.03–1.55 dorso-ventral diameters in length), lying along the right dorsal surface. There are two flattened dorsal lobes on the macronucleus, one at the anterior end and one in the middle. In dorsal view the macronucleus is seen to be curved, with the convex side to the right. The micronucleus is a small, ellipsoidal body, from 4 to 8μ in diameter, lying between the two lobes of the macronucleus. The conjugation of *O. gracile* has been described by Dogiel (1925*c*).

Two contractile vacuoles are present, lying close against the left dorsal edge of the macronucleus, one vacuole in the depression behind each lobe of the macronucleus. The canals leading to the surface are relatively long. Dogiel (1927) figures *O. gracile gracile* with only two vacuoles, but in his description states that it may have three. We have found that all the individuals of this species which possess more than two vacuoles are division stages, as is shown clearly by the rudiments of the membranelle zones of the posterior daughter. Another species, *O. trivesiculatum*, is similar in many repects to *O. gracile*, but has three vacuoles. Since Dogiel states that he has found the· "race" with three vacuoles dominating in some hosts, it is probable he was dealing with both species.

The mouth opens into the short, tubular gullet, the fibrils spreading out posteriorly and becoming pressed against the ectoplasm of the right side, where they lie in the region between the anterior third of the right side of the endoplasmic sack and its posterior end. Some of the fibrils extend beneath the macronucleus and curve dorsally and to the right across the posterior part of the dorsal surface.

Food.—The food consists of relatively large pieces of plant débris.
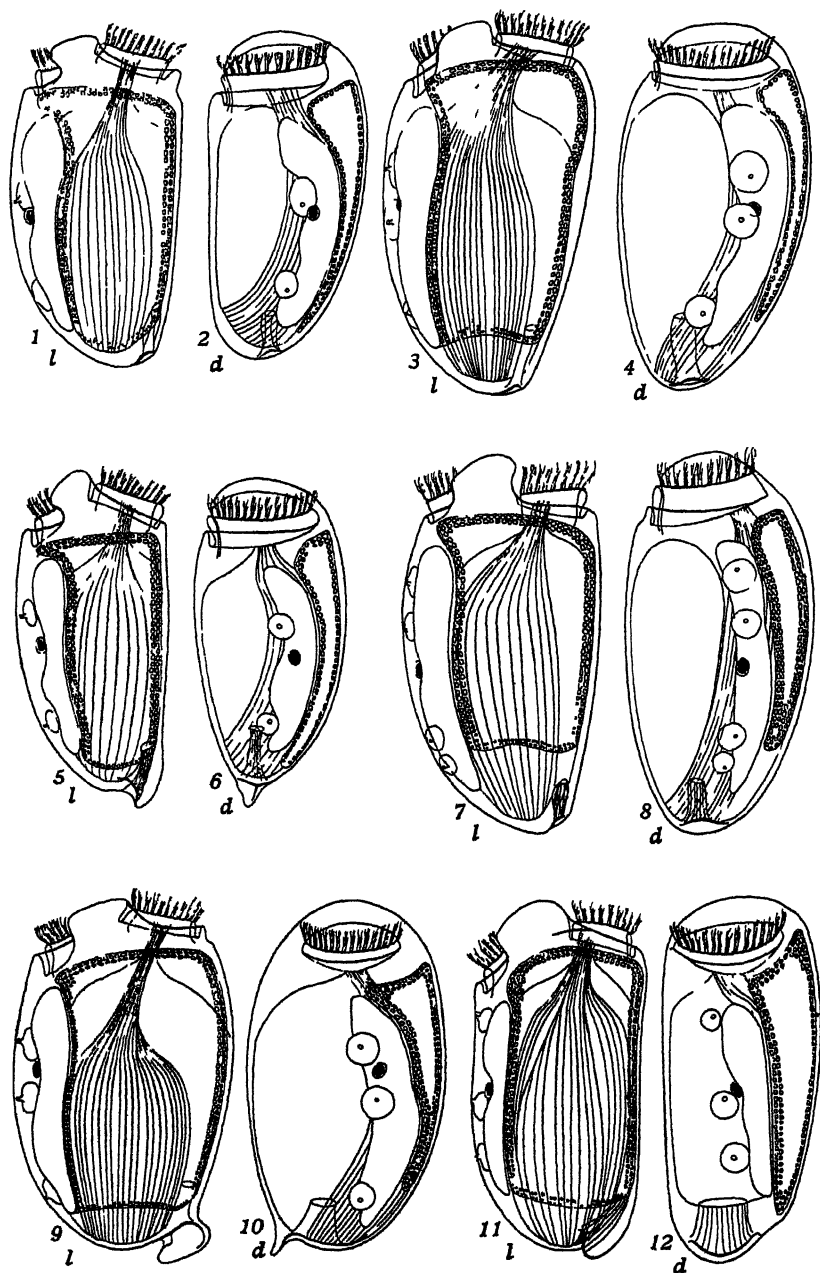
Fig. J.   1 and 2, *Ostracodinium gracile* (Dogiel 1925); 3 and 4, *O. trivesiculatum* sp. nov.; 5 and 6, *O. venustum* sp. nov.; 7 and 8, *O. quadrivesiculatum* sp. nov.; 9 and 10, *O. clipeolum* sp. nov.; 11 and 12, *O. rugoloricatum* sp. nov. *l*, right lateral view; *d*, dorsal view.   ×500.

Measurements.—The following measurements were taken from 10 individuals taken at random from *Bos indicus:*

Axis	Microns	Proportional
Length 101	(92–125)	1.88 (1.75–2.16)
Dorso-ventral diameter 54	(42– 60)	1.00
Transverse diameter 50	(40– 55)	0.94 (0.88–0.98)
Macronucleus 64	(54– 90)	1.19 (1.03–1.55)
Mouth 19	(15– 22)	0.35 (0.29–0.43)

The measurements reported by Dogiel (1927) give a somewhat wider range in size (90–133μ), and 2.0 dorso-ventral diameters in length.

Occurrence.—*Ostracodinium gracile* has been reported from cattle and sheep in the U. S. S. R. and from antelope (*Bubalis cokei, Aepyceros melampus,* and *Madoqua* sp.) from British East Africa by Dogiel (1927). It was found in four of the *Bos indicus* examined from Coonoor, India, and in three from Colombo, Ceylon.

Relationships.—*Ostracodinium gracile* is closely allied to *O. trivesiculatum, O. quadrivesiculatum,* and *O. tenue* in its triangular shape, size, and proportions of the body, and in the lack of spines. It is also closely related in shape and proportions to two-spined species, *O. nanum,* and *O. gladiator.*

Ostracodinium tenue (Dogiel 1925)

Diplodinium gracile forma *tenue* Dogiel 1925*b*, p. 129, fig. 5; Fantham 1926, p. 568.
Ostracodinium gracile forma *tenue,* Dogiel 1927, p. 146, fig. 81*c.*

Diagnosis.—Body slender (2.12 dorso-ventral diameters in length) ; skeletal plate extends across right surface; macronucleus with an anterior and a median dorsal lobe; two contractile vacuoles; posterior end smoothly rounded. Length 59–76μ.

Occurrence.—*O. tenue* is reported by Dogiel (1927) from the antelope (*Bubalis cokei*) from British East Africa; and from cattle from South Africa by Fantham (1928).

Relationships.—*O. tenue* is the smallest species of a group including *O. gracile, O. trivesiculatum,* and *O. quadrivesiculatum.* It is most closely related in morphology to *O. gracile* and the two are most clearly separated by their difference in size. Among the small, spined species, *O. nanum* is the closest to *O. tenue.*

Ostracodinium trivesiculatum sp. nov.

Plate 7, figure 22; figure J, 3, 4

Diagnosis.—Body triangular in lateral view (1.67–2.34 dorso-ventral diameters in length) ; skeletal plate extends across right side; macronucleus with a small, shallow depression in the middle of the left side; three contractile vacuoles; posterior end smoothly rounded. Length 78–100μ, 10 specimens.

Description.—*Ostracodinium trivesiculatum* is similar to *O. gracile* in general shape. It is triangular in lateral view. It is slightly com-

pressed laterally to 0.90–1.00 dorso-ventral diameters. The ventral and left sides are nearly flat and the dorsal and right sides are strongly convex. The membranelle zones form the base of the triangle. The posterior end is smoothly rounded.

The oral area is relatively large (0.37–0.40 dorso-ventral diameters in diameter). It is inclined ventrally at an angle of 10°–20°, but is not inclined to the left or right. The dorsal membranelle region is somewhat smaller than in *O. gracile*. The operculum is fairly prominent.

The broad skeletal plate extends laterally from the macronucleus to the ventral surface.

The macronucleus is a long, rod-like body (1.08–1.43 dorso-ventral diameters in length), lying along the right dorsal surface. There is a small, shallow depression in the middle of the left side and occasionally in the posterior end. The macronucleus is curved parallel to the curvature of the right side. The relatively smooth outline of the macronucleus of *O. trivesiculatum* is in sharp contrast to the lobed macronucleus of *O. gracile*. The micronucleus is a small, ellipsoidal body, from 3 to 6μ in length, lying in a small depression in the middle of the dorsal surface of the macronucleus.

There are three contractile vacuoles, one just anterior to the micronucleus, one just posterior to it, and one vacuole at the level of the posterior end of the macronucleus. The two anterior vacuoles are at the right of the dorsal mid-line, the posterior vacuole is under the mid-line. The excretory canals open from the middle of the vacuoles.

The mouth opens into the narrow, tubular gullet. The oesophageal fibrils arise in the gullet, spread out in the anterior third of the endoplasmic sack and press close against the boundary layer in the posterior two-thirds of the sack, along the right and dorsal sides.

The rectum is a narrow cylinder lying in the ectoplasm at the postero-ventral side of the body. The small, circular anus lies in the ventral part of the posterior end.

Food.—The food consists of large bits of plant débris.

Variations.—*Ostracodinium trivesiculatum* is usually very markedly triangular in side view, but in many specimens the posterior end is more bluntly rounded.

Measurements.—The following measurements were made from 10 specimens picked at random from *Bos indicus:*

Axis	Microns	Proportional
Length	98 (78–100)	1.94 (1.67–2.34)
Dorso-ventral diameter	50 (42– 60)	1.00
Transverse diameter	48 (41– 56)	0.95 (0.90–1.00)
Macronucleus	61 (50– 73)	1.24 (1.08–1.43)
Mouth	20 (18– 22)	0.39 (0.37–0.43)

Occurrence.—*Ostracodinium trivesiculatum* was found in three of the *Bos indicus* examined from Coonoor, India, and in three from Colombo, Ceylon.

Relationships.—*O. trivesiculatum* is a member of a group including *O. gracile, O. tenue,* and *O. quadrivesiculatum.* It differs from *O. gracile* chiefly in the presence of three, instead of two vacuoles.

Ostracodinium quadrivesiculatum sp. nov.

Plate 7, figure 19; figure J, 7, 8

Diagnosis.—Body triangular in side view (1.96–2.24 dorso-ventral diameters in length); skeletal plate extends across right side; macronucleus an elongate rod-like body; four contractile vacuoles; posterior end bluntly pointed, no lobes or spines present. Length 92–112μ, 10 specimens.

Description.—*Ostracodinium quadrivesiculatum* is triangular in side view, 1.96–2.24 dorso-ventral diameters in length. It is only slightly compressed laterally, the transdiameter averaging 0.96 dorso-ventral diameters. The ventral side is flat or slightly convex, the dorsal surface is strongly convex. The left surface is only slightly convex, the right surface is strongly so. The posterior end is smoothly rounded.

The oral area is 0.36–0.42 dorso-ventral diameters in diameter. It is not inclined ventrally, but is inclined to the left at an angle of 10°–15°. The dorsal membranelle zone is relatively large. The operculum is prominent and extends anteriorly considerably beyond the adoral lips.

The ectoplasm is thin and is thickened only in the region of the skeletal plate, nuclei, and rectum. The skeletal plate extends laterally from the right surface of the macronucleus to the ventral surface. It extends posteriorly to the posterior quarter of the body.

The macronucleus is an elongate, rod-like body, 1.15–1.59 dorso-ventral diameters in length. It lies beneath the right dorsal surface. There is a small, shallow depression in the middle of the dorsal surface. The micronucleus is small and ellipsoidal, from 4 to 6μ in diameter. It lies in a small depression in the middle of the left dorsal surface of the macronucleus. There are four contractile vacuoles lying along the left dorsal surface of the macronucleus. One pair lies just anterior to the micronucleus, the other pair lies near the posterior end of the macronucleus. The excretory canals are relatively small.

The narrow, tubular gullet extends posteriorly and to the right, expanding to form the oesophagus in the anterior third of the endoplasmic sack, in which region the fibrils come to lie close against the boundary layer. These fibrils are most clearly visible under the posterior two-thirds of the right and dorsal sides of the boundary layer.

The rectum is a narrow, cylindrical structure lying in the posteroventral side of the body. The elliptical anus lies in the posterior end of the body, near the ventral surface.

Food.—The food consists of large bits of plant débris.

Measurements.—The following measurements were made from 10 individuals picked at random from *Bos indicus:*

Axis	Microns	Proportional
Length	102 (92–112)	2.10 (1.96–2.24)
Dorso-ventral diameter	49 (43– 56)	1.00
Transdiameter	47 (42– 50)	0.96 (0.92–1.02)
Macronucleus	67 (52– 80)	1.38 (1.15–1.59)
Mouth	19 (18– 20)	0.39 (0.36–0.42)

Occurrence.—Ostracodinium quadrivesiculatum was present in small numbers in two of the *Bos indicus* from Coonoor, India, and in two from Colombo, Ceylon.

Relationships.—O. quadrivesiculatum is a member of a group including *O. gracile* and *O. trivesiculatum.* In these latter species two and three vacuoles, respectively, are present, while in *O. quadrivesiculatum*, four vacuoles are present. A small species, *O. tenue*, with two vacuoles, also belongs in this group because of the triangular shape of the body and the shape of the macronucleus and skeletal plate.

Ostracodinium nanum (Dogiel 1925)

Diplodinium gracile forma *nanum* Dogiel 1925b, p. 129, fig. 5; Fantham 1926, p. 568.
Ostracodinium gracile forma *nanum* Dogiel 1927, pp. 147–148, fig. 81a.

Diagnosis.—Body relatively short (1.64 dorso-ventral diameters in length); skeletal plate extends between the macronucleus and ventral surface; short, stout macronucleus; two small contractile vacuoles; a short, slender, ventral spine. Length 47–70μ.

Occurrence.—O. nanum is reported by Dogiel 1927 from antelopes (*Bubalis cokei* and *Madoqua* sp.) from British East Africa, and by Fantham (1926) from South African cattle.

Relationships.—O. nanum is closely related to *O. gladiator* in size and morphology, particularly by the presence in both of a slender ventral spine. *O. tenue*, among the spineless forms, is most nearly related to *O. nanum*.

Ostracodinium gladiator (Dogiel 1925)

Diplodinium gracile forma *gladiator* Dogiel 1925b, p. 130, fig. 5; Fantham 1926, p. 568, fig. 7.
Ostracodinium gracile forma *gladiator* Dogiel 1927, pp. 146–147, fig. 81b.

Diagnosis.—Body slender (2.1 dorso-ventral diameters in length); skeletal plate extends between the macronucleus and ventral side; macronucleus with dorsal lobe on anterior end; two contractile vacuoles; a long, very narrow, ventral spine. Length 78–112μ.

Occurrence.—O. gladiator is reported by Dogiel (1927) from antelopes (*Bubalis cokei* and *Madoqua* sp.) from British East Africa, and by Fantham (1926) from South African cattle.

Relationships.—O. gladiator is a relatively large species similar to the smaller *O. nanum. O. gladiator* is very similar in shape and morphology to *O. gracile*, the main difference being the presence of a spine in the former.

Ostracodinium crassum (Dogiel 1925)

Diplodinium crassum Dogiel 1925b, p. 132, fig. 46; Fantham 1926, p. 568.
Ostracodinium crassum Dogiel 1927, pp. 142–143, fig. 80.

Diagnosis.—Body short and heavy (1.42 dorso-ventral diameters in length); skeletal plate extends under only one-half of the right side; macronucleus short and stout with a wide, shallow depression in anterior half of dorsal side; two contractile vacuoles; posterior end smoothly rounded. Length 120–142μ.

Occurrence.—*O. crassum* is reported by Dogiel (1927) from the steenbock (*Rhaphiceros* sp.) from British East Africa; and from South African cattle by Fantham (1926).

Relationships.—*O. crassum* is separated from the other species of *Ostracodinium* by its relatively short and broad body and by the narrowness of its skeletal plate.

Ostracodinium obtusum (Dogiel and Fedorowa 1925)

Diplodinium dentatum variety *obtusum* Dogiel and Fedorowa 1925, p. 101, fig. 5; Fantham 1926, p. 567.
Diplodinium obtusum Dogiel 1925e, pp. 53–55, pl. 2, fig. 13; 1926, p. 262.
Ostracodinium obtusum forma *obtusum* Dogiel 1927, pp. 136–137, fig. 76.
Diplodinium Hegneri Becker and Talbott, *partim*, 1927, p. 357, pl. 2, fig. 17.

Diagnosis.—Body an ellipsoid (2.1 dorso-ventral diameters in length), only slightly compressed laterally; posterior part of skeleton extends across only two-thirds of right side; macronucleus elongate and rod-like; six contractile vacuoles; posterior end smoothly rounded. Length 118–148μ.

Occurrence.—*O. obtusum* is reported by Dogiel (1927) from cattle and reindeer from various parts of the U. S. S. R.; from South African cattle by Fantham (1926); and by Becker and Talbott (1927) from cattle from Iowa.

Relationships.—*O. obtusum*, *O. monolobum*, and *O. dilobum*, form a fairly homogeneous group, similar in shape, size, and in the relatively large number of contractile vacuoles. *O. obtusum*, with a smoothly rounded posterior end, is the simplest of the group.

The description by Becker and Talbott (1927) of the "medium-sized individuals" of *Diplodinium Hegneri* and their figure shows that *Ostracodinium obtusum* comprised the major part of the specimens described as *D. Hegneri*. The rest of the "developmental cycle" of *D. Hegneri*, which they mention briefly, is evidently made up of several genera and species with various types of skeletal plates.

Ostracodinium venustum sp. nov.

Plate 7, figure 21; figure J, 5, 6

Diagnosis.—Body triangular in side view (1.76–2.06 dorso-ventral diameters in length); skeletal plate extends beneath right surface between macronucleus and ventral side; two dorsal lobes on macronucleus; two contractile vacuoles; ventral lobe small. Length 76–115μ, 10 specimens.

Description.—*Ostracodinium venustum* is triangular in side view, ellipsoidal in dorsal view. It is 1.72–2.06 dorso-ventral diameters in length, and laterally compressed (0.88–1.00 dorso-ventral diameters).

The oral area is 0.33–0.50 dorso-ventral diameters in diameter. It is inclined ventrally at an angle of 20°–40°, and to the left at an angle of 10°–20°. Both angles are greater than in any other species of *Ostracodinium* found in our material. The dorsal membranelle zone is well developed. The operculum is small and projects only a short distance anteriorly from the adoral zone.

The dorsal surface is convex with the greatest curvature in the middle. The ventral surface is nearly plane, but there is a slight convexity in the anterior third, and a very slight concavity in the posterior two-thirds. Both lateral surfaces are convex. There is a small caudal lobe from 3 to 7μ long, projecting from the postero-ventral end of the body slightly to the left of the mid-line.

The skeletal plate lies beneath the right surface, extending laterally between the macronucleus and the ventral side, and longitudinally between the membranelle zones and the posterior quarter of the body.

The macronucleus is an elongate body, 1.00–1.44 dorso-ventral diameters in length. It lies beneath the dorsal surface slightly to the right of the mid-line.

There are two well marked lobes extending dorsally and to the left from the macronucleus, one at the anterior end and one just behind the middle. The micronucleus lies on the dorsal side of the macronucleus just in front of the median lobe.

Two contractile vacuoles are present, lying dorsally and to the left of the macronucleus, one behind each dorsal lobe of the macronucleus. The excretory canals and pores open from the middle of each vacuole.

The short, tubular gullet extends posteriorly through the anterior quarter of the endoplasm. There the bundle of fibrils spreads out and comes to lie just beneath the skeletal plate. Some of the fibrils extend dorsally and to the left beneath the macronucleus, most of them extend beneath the right surface. All the fibrils terminate at the posterior end of the endoplasmic sack. The small, tubular rectum lies in the postero-ventral end of the body along the mid-line. The rectum is lined with fine, longitudinal fibrils. The circular anus opens just dorsal to the ventral lobe.

Food.—The food consists of large bits of plant débris.

Measurements.—The following measurements were made from 10 specimens picked at random from *Bos indicus* material:

Axis	Microns	Proportional
Length ..	95 (76–115)	1.88 (1.72–2.06)
Transdiameter	47 (37– 52)	0.93 (0.88–1.00)
Dorso-ventral diameter	51 (41– 60)	1.00
Macronucleus	59 (46– 76)	1.16 (1.00–1.44)
Mouth	21 (19– 25)	0.42 (0.33–0.50)
Ventral lobe	3 (2– 10)	0.07 (0.03–0.21)

Occurrence.—Ostracodinium venustum occurred in two of the *Bos indicus* from Coonoor, India, and in two from Colombo, Ceylon.

Relationships.—O. venustum is similar to *O. gracile* in shape, size, number of vacuoles, and shape of macronucleus. The small ventral lobe of *O. venustum* is the main morphological distinction between the two.

Ostracodinium dogieli nom. nov.

Ostracodinium gracile forma *monolobum* Dogiel, *partim*, 1927, pp. 148–149, fig. 82b (for p. 149, see *O. clipeolum*).

Diagnosis.—Body ellipsoidal (2.0 dorso-ventral diameters in length); dorsal surface strongly convex, ventral surface slightly convex; skeletal plate extends between macronucleus and ventral side; macronucleus with two dorsal lobes, one anterior and one median; two contractile vacuoles; ventral lobe laterally flattened and lies at the left of the anus. Length 92–130μ.

Occurrence.—O. dogieli is reported by Dogiel (1927) from cattle from the U. S. S. R.

Relationships.—O. dogieli is similar to *O. clipeolum* in proportions, size, and in the peculiar shape of the ventral lobe found in both species. The two species differ chiefly in the number of vacuoles and in the curvature of the surfaces of the body.

Dogiel (1927) gave the name *monolobum* to two different formas of *Ostracodinium* (*O. obtusum monolobum* and *O. gracile monolobum*). Article 11 of the International Rules of Nomenclature states: "Specific and subspecific names are subject to the same rules and recommendations, and from a nomenclatural standpoint they are coordinate, that is, they are of the same value." Since the original description of *O. gracile monolobum* is contained in a later page of Dogiel's monograph (1927) than that of *O. obtusum monolobum* the name as applied to the former is preoccupied and we therefore substitute *O. dogieli* for *O. gracile monolobum*.

Ostracodinium clipeolum sp. nov.

Plate 6, figure 15; figure J, 9, 10

Diagnosis.—Body ellipsoidal (1.64–2.14 dorso-ventral diameters in length); skeletal plate extends beneath right surface between the macronucleus and the ventral side; two dorsal lobes on macronucleus; three contractile vacuoles; a laterally flattened lobe projecting from the postero-ventral surface at left of the mid-line. Length 92–128μ, 10 specimens.

Description.—Ostracodinium clipeolum is ellipsoidal in general outline, 1.64–2.14 dorso-ventral diameters in length, and compressed

laterally to 0.91–0.98 dorso-ventral diameters. The oral area is 0.32–
0.38 dorso-ventral diameters in diameter. It is inclined ventrally at
an angle of about 20° and to the left at an angle of about 10° or less.
The dorsal membranelle zone is relatively inconspicuous and the lips
are small. The operculum is small and projects only a short distance
anteriorly from the adoral zone. The dorsal surface is convex, the
curvature increasing posteriorly and merging with the curvature of
the smoothly rounded posterior end. The anterior half of the ventral
surface is convex, the posterior half nearly plane. In *O. dogieli*, the
whole ventral surface is flat. In *O. clipeolum* both right and left
surfaces are convex and merge posteriorly with the rounded posterior
end of the body.

A small, laterally flattened, shield-shaped lobe lies on the postero-
ventral end of the body at the left of the anus. The concave side opens
to the right. The lobe lies at an angle of about 30°–45° with the
main axis of the body. The lobe varies from 0.11–0.26 dorso-ventral
diameters in length.

The skeletal plate extends laterally beneath the right surface from
the macronucleus to the ventral side. It extends longitudinally from
the base of the membranelle zones to the posterior end of the body.
It occupies nearly the whole of the right surface and is more extensive
than in many other species, such as *O. mammosum* and *O. crassum*.

The macronucleus is an elongate body, 1.00–1.50 dorso-ventral
diameters in length. Two flat lobes extend dorsally, one on the
anterior end of the macronucleus and one on the posterior half. The
macronucleus lies beneath the right dorsal surface, close against the
dorsal edge of the skeletal plate.

There are three contractile vacuoles present, lying along the dorsal
side of the macronucleus. Two vacuoles lie between the anterior and
posterior lobes, the third vacuole lies just behind the posterior lobe
of the macronucleus. The excretory canals open dorsally from the
middle of each vacuole.

The long, tubular gullet extends through the anterior third of the
endoplasm, then expands, and forms the oesophagus, with the fibrils
lying close against the boundary layer beneath the right surface. Some
of the fibrils curve dorsally and extend posteriorly beneath the macro-
nucleus and posterior contractile vacuole to the end of the body.

The small, cylindrical rectum lies in the mid-line of the postero-
ventral side of the body and opens by the small, elliptical anus.

Food.—The food consists of relatively large bits of plant débris.

Variations.—The posterior lobe is often reduced, and may be little
more than a semicircular flap. It is, however, always strongly com-
pressed laterally and is always found at the left of the mid-line.

Measurements.—The following measurements were made from 10
specimens picked at random from *Bos indicus:*

Axis	Microns		Proportional	
Length	106	(92–128)	1.88	(1.64–2.14)
Transdiameter	53	(48– 60)	0.94	(0.91–0.98)
Dorso-ventral diameter	57	(50– 65)	1.00	
Macronucleus	70	(60– 90)	1.24	(1.00–1.50)
Oral area	20	(18– 22)	0.35	(0.32–0.38)
Caudal lobe	10	(6– 16)	0.17	(0.11–0.26)

Occurrence.—Ostracodinium clipeolum occurred in four of the *Bos indicus* examined from Coonoor, India, and in three from Colombo, Ceylon.

Relationships.—O. clipeolum resembles *O. dogieli* in proportions, size, and in the shape of the caudal lobe.

Ostracodinium monolobum Dogiel 1927

Ostracodinium obtusum forma *monolobum* Dogiel 1927, pp. 137–138, fig. 77.

*Diagnosis.—*Body rectangular in side view (1.9 dorso-ventral diameters in length); skeletal plate extends under only two-thirds of dorsal side; macronucleus elongate and rod-like; five contractile vacuoles; large ventral lobe. Length 105–150μ.

Occurrence.—O. monolobum is reported by Dogiel (1927) from cattle from Leningrad and Vladivostok, U. S. S. R.

Relationships.—O. monolobum is related in size, proportions, number of vacuoles, and shape of macronucleus to *O. obtusum* and *O. dilobum.*

Ostracodinium dilobum Dogiel 1927

Diplodinium gracile forma *dentatum* Fantham 1926, p. 568.
Ostracodinium obtusum forma *dilobum* Dogiel 1927, p. 139, fig. 78a, b.

*Diagnosis.—*Body ellipsoidal (1.8 dorso-ventral diameters in length); macronucleus elongate and rod-like; five conctractile vacuoles; skeletal plate extends under only two-thirds of dorsal side; dorsal lobe laterally compressed; ventral lobe dorso-ventrally compressed. Length 88–140μ.

Occurrence.—O. dilobum is reported by Dogiel (1927) from cattle from Leningrad and Vladivostok, U. S. S. R.

Relationships.—O. dilobum is related to *O. obtusum* and *O. monolobum* in size, proportions, and in the shape of the macronucleus. The two caudal lobes are much like those of *O. mammosum.* The ventral lobe is dorso-ventrally compressed and in this way resembles the ventral lobe of *O. mammosum,* but it is not scoop-shaped as in the latter species. The dorsal lobes of the two are similar.

Fantham (1926) was the first to describe this species, but his name *D. gracile dentatum* was preoccupied by *D. dentatum* Fiorentini 1889. According to Article 11 of the International Rules of Nomenclature, Fantham's name must be rejected and the name given by Dogiel retained.

Ostracodinium rugoloricatum sp. nov.

Plate 7, figure 20; figures I, 1; J, 11, 12

*Diagnosis.—*Body rectangular in outline (1.78–2.16 dorso-ventral diameters in length); dorsal side of skeletal plate turns in and extends toward the middle of the body; rectum wide, strongly compressed

dorso-ventrally; macronucleus straight and rod-like; three contractile vacuoles; a flattened ventral lobe. Length 84–125μ, 10 specimens.

Description.—*Ostracodinium rugoloricatum* is ellipsoidal in general appearance (1.78–2.16 dorso-ventral diameters in length), with both ends bluntly rounded, and the sides nearly plane. The lateral compression varies from 0.80–1.00 dorso-ventral diameters.

The oral area is 0.32–0.36 dorso-ventral diameters in diameter and is somewhat smaller than in the *O. gracile* group. It is inclined ventrally at an angle of about 30° and to the left at an angle of about 10°. The dorsal membranelle zone is relatively small. The operculum is relatively large and overhangs the dorsal zone. A distinct cuticular fold connects the outer lips of the dorsal and adoral zones along the left side.

The anterior three-quarters of the ventral surface is flat or slightly concave. The posterior quarter is convex and forms the ventral lobe. The anterior half of the dorsal surface is flat or slightly concave, the posterior half strongly convex. The left surface is flat, the right surface convex. There is a wide, flattened ventral lobe from 2 to 6μ in length, on the ventral third of the posterior end of the body.

The ectoplasm on the right and posterior sides is relatively thin, but is much thickened in the regions of the nuclei and the skeletal plate. The boundary layer is clearly visible.

The skeletal plate extends laterally from the macronucleus to the ventral side of the body. The dorsal edge of the plate folds inward near the macronucleus and extends toward the middle of the body (fig. I, 1). The skeletal plate in most species of *Ostracodinium* terminates near the macronucleus and turns in very little, if any (fig. I, 2).

The macronucleus is a straight, narrow, rod-like body, 1.08–1.48 dorso-ventral diameters in length, lying under the right dorsal edge of the body. There is a deep depression in the middle of the left dorsal side of the macronucleus in which the micronucleus lies. The micronucleus is a small, ellipsoidal body, from 3 to 6μ in length.

There are three contractile vacuoles lying along the left dorsal edge of the macronucleus. One vacuole is at the level of the anterior edge of the macronucleus, one vacuole is just behind the micronucleus, and one near the level of the posterior end of the macronucleus. The canals leading to the surface are relatively short.

The narrow tubular gullet extends posteriorly and expands to occupy the space enclosed by the skeletal plate. The heavy oesophageal fibrils arising from the gullet pass from the anterior end of the endoplasmic sack to its posterior end, and laterally between the macronucleus and the ventral edge of the body. The fibrils do not extend beneath the dorsal surface as they do in the *O. gracile* group.

The rectum is a wide, dorso-ventrally compressed structure lying beneath the ventral lobe and parallel to it. It is lined by fine, parallel fibrils extending longitudinally. It opens to the exterior by the thin, slit-like anus.

Food.—The food consists of small bits of plant débris.

Measurements.—The following measurements were made from 10 individuals picked at random from *Bos indicus:*

Axis	Microns	Proportional
Length	100 (84–125)	2.06 (1.78–2.16)
Dorso-ventral diameter	48 (37– 58)	1.00
Transdiameter	45 (33– 57)	0.94 (0.80–1.00)
Macronucleus	60 (40– 80)	1.26 (1.08–1.48)
Mouth	16 (13– 20)	0.35 (0.32–0.36)
Ventral lobe	4 (2– 6)	0.08 (0.04–0.12)

Relationships.—The exceptionally large inturned skeletal plate, the ventral lobe, and dorso-ventrally compressed rectum and anus separate *O. rugoloricatum* from the other species of the genus.

Ostracodinium ventricosum (Buisson 1923)

Diplodinium ventricosum Buisson 1923*a*, pp. 157–158, fig. 6; Dogiel 1925*b*, pp. 132, 141.

Ostracodinium ventricosum forma *ventricosum* Dogiel 1927, pp. 149–150, fig. 83.

Diagnosis.—Body short (1.27 dorso-ventral diameters in length); skeletal plate extends under only two-thirds of dorsal side; macronucleus short; a single, stout, ventral spine. Length 60–80μ.

Occurrence.—*O. ventricosum* is reported by Buisson (1923*b*) from an antelope (*Bubalis lichtensteini*) from Belgian Congo.

Relationships.—The descriptions of *O. ventricosum*, *O. dyurum*, *O. stokyi*, and *O. crustaceum* are insufficient to form the basis of a discussion of their morphological relations to the other species of the genus.

Ostracodinium stokyi (Buisson 1924)

Diplodinium stokyi Buisson 1924, p. 158, fig. 8; Dogiel 1925*b*, p. 141; Dogiel and Fedorowa 1925, p. 101.

Ostracodinium stokyi Dogiel 1927, p. 151, fig. 85.

Diagnosis.—Body short and broad; skeleton extends under only one-half of right side; macronucleus small and rod-like; two (?) contractile vacuoles; two ventral spines. Length 70–105μ.

Occurrence.—*O stokyi* is reported by Buisson (1924) from the antelope (*Hippotragus equinus*) from Belgian Congo.

Ostracodinium dyurum (Buisson 1924)

Diplodinium ventricosum variety *dyurum* Buisson 1924, p. 158, fig. 7.

Ostracodinium ventricosum forma *dyurum* Dogiel 1927, pp. 150–151, fig. 84.

Diagnosis.—Body short and broad; skeletal plate extends under only two-thirds of right side; macronucleus short; ventral spine curves dorsally, dorsal spine straight. Length (no measurements given).

Occurrence.—*O. dyurum* is reported by Buisson (1924) from the antelope (*Bubalis lichtensteini*) from Belgian Congo.

Ostracodinium crustaceum (Buisson 1924)

Diplodinium crustaceum Buisson 1924, p. 158, fig. 9.
Ostracodinium crustaceum Dogiel 1927, p. 152, fig. 86.

Diagnosis.—Body long and slender (2.7 dorso-ventral diameters in length); skeletal plate extends beneath right surface between the macronucleus and ventral side; macronucleus elongate and rod-like; two contractile vacuoles; posterior end rounded. Length 136μ.

Occurrence.—*O. crustaceum* is reported by Buisson (1924) from antelopes (*Cephalophus grimmia* and *Tragelaphus scriptus*) from Belgian Congo.

Species inquirendae—

Diplodinium gracile forma diverticulatum Fantham 1926

Fantham, 1926, p. 568.

Fantham's description states merely that this is a form of *Diplodinium gracile,* with "pocket-like expansions on the ventral side."

Enoploplastron gen. nov.

Figure H, 1

Ostracodinium Dogiel, *partim,* 1927, pp. 152–155, figs. 87–88 (for pp. 134–152, figs. 76–86, see *Ostracodinium*).

Diagnosis.—Ophryoscolecidae with dorsal and adoral membranelle zones near anterior end of body; three skeletal plates beneath right and ventral surfaces of body, either separate or partly fused; two contractile vacuoles; oesophageal fibrils heavy.

Type species.—*Enoploplastron triloricatum* (Dogiel 1925) from cattle from U. S. S. R.

Dogiel (1927) states that the skeleton is one of the most important characters involved in a systematic analysis of the family. In the same paper he includes in his subgenus *Ostracodinium* the species we have included in *Enoploplastron,* although he emphasizes the fact that they are separated from the rest of the species of *Ostracodinium* by very great differences in skeletal structure. In order to unify *Ostracodinium,* we retain in it only those species with a single, broad skeletal plate, and those species with three narrow skeletal plates are placed by us in *Enoploplastron.*

<center>CHARACTERS OF SYSTEMATIC IMPORTANCE</center>

The three narrow skeletal plates of *Enoploplastron* separate it from *Ostracodinium* which has a single, broad skeletal plate. The dorsal and median skeletal plates of *Enoploplastron* lie beneath the right side of the body between the macronucleus and the ventral surface. The ventral plate lies adjacent to the median plate and extends beneath the ventral surface. The median plate is slightly larger than the other two. The plates lie close together with their edges touching, except in the middle of the anterior half of the body. In this region the plates are separate, leaving two windows of plain ectoplasm between the plates. This arrangement of plates is similar to that in *Epidinium* and *Ophryoscolex*, except that in the latter genera a window is found only between the dorsal and median plates.

Other characters of *Enoploplastron* link it to *Ostracodinium*. The dorsal membranelle zone is near the anterior end of the body, instead of distinctly behind it as in *Epidinium*. The oesophageal fibrils are like those of *Ostracodinium*. The food ingested consists of large pieces of plant débris as in *Ostracodinium* and is a marked contrast to the bacterial clumps ingested by *Epidinium*.

The two contractile vacuoles, the rod-like macronucleus, the ectoplasm, etc., show no differences from those structures in other genera such as *Eremoplastron*, *Ostracodinium*, and *Epidinium*, and so are less important in generic classification.

<center>**Enoploplastron triloricatum** (Dogiel 1925)</center>

<center>Figure H1</center>

> *Diplodinium triloricatum* Dogiel 1925b, pp. 133, 141, fig. 6; 1925a, pp. 292,
>   326, 347, pl. 18, figs. 65–76; Fantham 1926, p. 568.
> *Diplodinium triloricatum* forma *triloricatum* Dogiel 1925e, p. 56, fig. 16.
> *Ostracodinium triloricatum* forma *triloricatum* Dogiel 1927, pp. 152–154,
>   fig. 87.
> *Diplodinium ecaudatum* Rees, 1930, pp. 369–370.

Diagnosis.—Body ellipsoidal (1.9 dorso-ventral diameters in length); skeletal plates separate; macronucleus with a shallow depression in anterior half of dorsal side; posterior end smoothly rounded. Length 60–112μ.

Occurrence.—*Enoploplastron triloricatum* is reported by Dogiel (1927) from cattle from U. S. S. R., from reindeer (*Rangifer tarandus*) from northern U. S. S. R., and from antelope (*Rhaphiceros* sp.) from British East Africa; by Fantham (1926) from cattle from South Africa; by Rees (1930) from Louisiana.

Rees (1930) described in a short note a ciliate which he identified as *Diplodinium* (now *Epidinium*) *ecaudatum*. He emphasized in his description of this ciliate the heavy oesophageal fibrils and also the ingestion of large pieces of plant débris. Since the oesophageal fibrils of *Epidinium* are relatively thin, and since it rarely, if ever, ingests anything but very small material, such as bacteria, it is evident that Rees was not dealing with *Epidinium ecaudatum*.

Enoploplastron triloricatum has three skeletal plates, ingests large pieces of plant débris, has prominent oesophageal fibrils, and fits the description given by Rees.

Enoploplastron confluens (Dogiel 1925)

Diplodinium triloricatum forma *confluens* Dogiel 1925e, p. 56, fig. 17.
Ostracodinium triloricatum forma *confluens* Dogiel 1927, pp. 154–155, fig. 88.

Diagnosis.—Body ellipsoidal (1.6 dorso-ventral diameters in length) ; skeletal plates fused except in the anterior third of the body; macronucleus with shallow depression in middle of dorsal side; posterior end smoothly rounded. Length 120–157μ.

Occurrence.—*Enoploplastron confluens* is reported by Dogiel (1927) from reindeer (*Rangifer tarandus*) from northern U. S. S. R.

SUMMARY

1. The type species of *Diplodinium* is *D. dentatum* (Stein) Schuberg.

2. The generic classification given in this paper is based on position and size of membranelle zones, number and form of skeletal plates, shape of the macronucleus, morphology of the oesophageal fibrils, along with other characters of the internal structures.

3. *Diplodinium* is restricted and the following genera recognized: *Diplodinium* s. str., *Eodinium* gen. nov., *Eremoplastron* gen. nov., *Eudiplodinium* Dogiel emended, *Diploplastron* gen. nov., *Metadinium* Awerinzew and Mutafowa, *Polyplastron* Dogiel emended, *Elytroplastron* gen. nov., *Ostracodinium* Dogiel emended, *Enoploplastron* gen. nov.

4. The material from *Bos indicus* contains twenty-one species of the above genera distributed as follows: two new species of *Eodinium*, four species (including one new species) of *Diplodinium*, five species (including three new species) of *Eremoplastron*, one species of *Eudiplodinium*, one species of *Metadinium*, seven species (including five new species) of *Ostracodinium*, and one species of *Elytroplastron*.

5. The fauna of the *Bos indicus* from Coonoor, India, showed no important differences from the fauna of the *Bos indicus* from Colombo,

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EXPLANATION OF PLATES

All figures are from whole mounts stained with chlor-zinc-iodide and drawn with camera lucida, × 750 unless otherwise stated.

PLATE 4
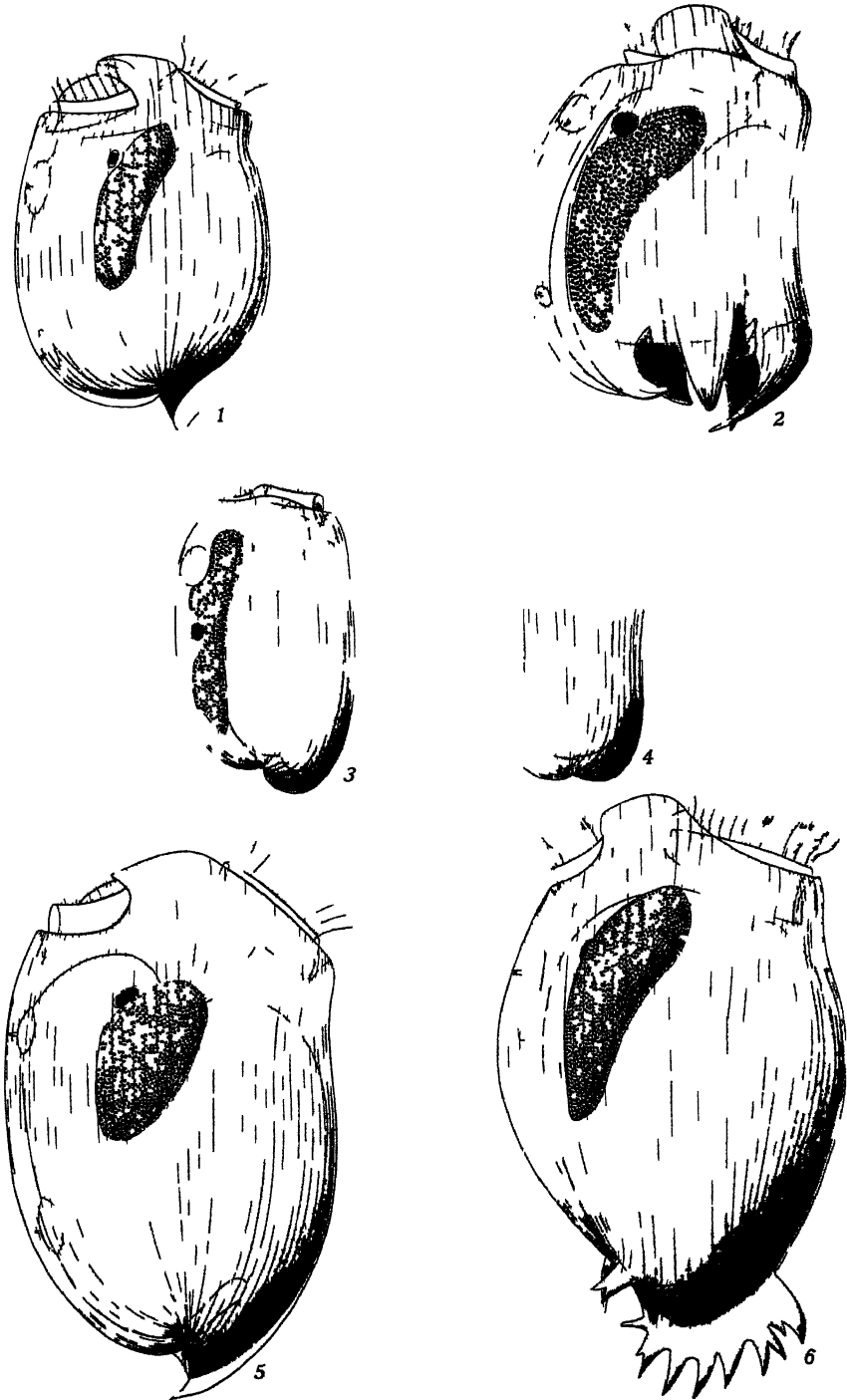
PLATE 5

PLATE 6

Fig. 13. *Elytroplastron bubali* (Dogiel 1928). × 500, left lateral view.
Fig. 14. *Elytroplastron bubali* (Dogiel 1928). × 500, right lateral view.
Fig. 15. *Ostracodinium clipeolum* sp. nov. × 500.
Fig. 16. *Metadinium medium* Awerinzew and Mutafowa 1914. × 375.

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PLATE 7

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GIGANTOMONAS LIGHTI SP. NOV.
A TRICHOMONAD FLAGELLATE FROM
KALOTERMES (PARANEOTERMES)
SIMPLICICORNIS BANKS

BY

FRANK H. CONNELL

GIGANTOMONAS LIGHTI SP. NOV., A TRICHO-MONAD FLAGELLATE FROM KALOTERMES (PARANEOTERMES) SIMPLICICORNIS BANKS

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The complex morphology, wide distribution, and marked differentation of cell organelles at division have long made trichomonad flagellates favorites for study. In spite of the large amount of work expended, numerous contradictions pertaining to their morphology and life-cycle occur in literature. *Gigantomonas lighti* sp. nov., the largest and most highly evolved member of this group, is remarkably well adapted for the study of many of the more troublesome points of trichomonad morphology.

The nearest relatives of this flagellate are found in the subfamily Devescovininae Kirby 1931. *Gigantomonas herculea* Dogiel 1916 from an African termite, *Hodotermes mossambicus* Hagen, *G. pulchra* (Grassi 1917) from a Chilean termite, and *Glyptotermes parvulus* Sjöstedt are the only other described species of this genus. *G. lighti* is found in the hind gut of *Kalotermes (Paraneotermes) simplicicornis* Banks, a termite from California and Arizona. Associated with *G. lighti* in the intestine of *K. simplicicornis* are *Hoplonympha natator* Light 1926, *Kofoidia loriculata* Light 1927, *Spirotrichonympha polygyra* Cupp 1930, and *Oxymonas dimorpha* Connell 1930, as well as undescribed species of *Spironympha*, *Trichomonas*, and *Janickiella*.

This study was made in the Zoological Laboratory of the University of California at Berkeley. To Professor S. F. Light, for whom this new species is named, I am deeply indebted for the abundance of living material used in this and other studies. I also owe thanks to Professors C. A. Kofoid, J. A. Long, and Harold Kirby, Jr., for much advice and helpful criticism.

MATERIAL

Several colonies of *Kalotermes* (*Paraneotermes*) *simplicicornis* Banks were made available by Professor Light at various times during the past three years through the courtesy of the Termite Investigations Committee. The termites were sent to me in the wood in which they were found and were kept in the laboratory without being disturbed until just before use. Most of this material came from the desert region near Indio, California, which is just north of the Salton Sea. In addition to the colonies taken in this region, one large colony was collected in Sabino Canyon, Arizona.

TECHNIQUE

At least four or five days before they are to be used the termites should be removed from the wood and placed upon a filter-paper diet. They may be kept successfully for some time in a moist chamber like that devised by Light and Andrews (1928). By such treatment, opaque wood particles are gradually eliminated from the gut and the quantity of intestinal fluid, never abundant in *K. simplicicornis,* is considerably augmented.

Living preparations were made by teasing out the contents of the gut upon a dry slide and applying a coverslip directly to the drop. When observations were to be prolonged, a drop of a 50–50 mixture of 67 per cent Locke's solution and the white of a fresh egg was applied to the edge of the coverslip, capillary attraction quickly carrying the fluid to and around the intestinal contents. Such preparations may be studied for an hour or more without their showing appreciable distortion or mortality.

In order to avoid undue distortion of *Gigantomonas,* intestinal smears must be made with considerable care. The gut was withdrawn from the termite with fine forceps and placed in the center of a clean slide. Then, working with two needles, the wall of the gut was punctured, allowing the intestinal fluid to flow out upon the slide. The gut was then drawn lightly through the drop in all directions making a thin, even smear. Sections of the gut had no advantages over smears.

For fixation the fluids of Schaudinn, Bouin, Flemming, Zirkle, and osmic vapor were used, the first being used hot (65° C) as well as cold.

Zirkle's copper dichromate mixture was used at the suggestion of Dr. Kirby. Though originally developed as a mitochondrial and nuclear fixative for plant tissue, it promises to be of considerable value as a general fixative for Protozoa. Zirkle's original formula appeared in Science, 66:400, and is reproduced below with the time changes that were found to be most favorable for fixation of *Gigantomonas*.

ZIRKLE'S COPPER DICHROMATE MIXTURE

Copper dichromate	5 grms.
Cupric oxide	1 grm.
10 per cent sol. acetic acid	1 c.c.
Distilled water	200 c.c.

Make up twenty-four hours before using. Allow cupric oxide to settle. Shake frequently. Zirkle recommends fixing plant tissue from thirty-six hours to six days. For Protozoa two days appear to be sufficient. Wash with 70 per cent alcohol.

Heidenhain's iron haematoxylin, Delafield's haematoxylin, safranin, Feulgen's nucleal reaction, and Mallory's triple connective stain were used with varying degrees of success. Eosin, erythrosin, and light green were occasionally employed as counter-stains though their value is questionable.

In studying the fate and development of organelles, it was soon found that no one combination of fixative and stain gave entirely satisfactory results for all organelles. For example, Schaudinn's fluid and Heidenhain's iron haematoxylin are satisfactory for most of the organelles but quite poor for the parabasal body. However, if the same fixative is used with Delafield's haematoxylin, the parabasal body stains beautifully but the paradesmose stains scarcely at all. Osmic vapor gives good results for study of the parabasal body when followed by Heidenhain's iron haematoxylin but this method results in very poor preparations of nuclei and mitotic figures.

Mallory's triple connective stain is difficult to handle with consistent results but often gives exceptional preparations of organelles and cytoplasmic inclusions.

Feulgen's nucleal reaction always stains the nucleus of *Gigantomonas* though it fails to color the nucleus of *Oxymonas* in the same preparations.

For flagella nothing equals the effect obtained with osmic iodide fixation followed by Ehrlich's haematoxylin. The flagella appear swollen, heavily stained, and stand out prominently and unmistakably.

GENERAL MORPHOLOGY

Observations on Living Material

In shape *Gigantomonas lighti* is roughly pyriform and drawn out posteriorly at the point where the axostyle leaves the body (pl. 8, fig. 1). The activity of the undulating membrane and cresta continually distort the external symmetry of the organism.
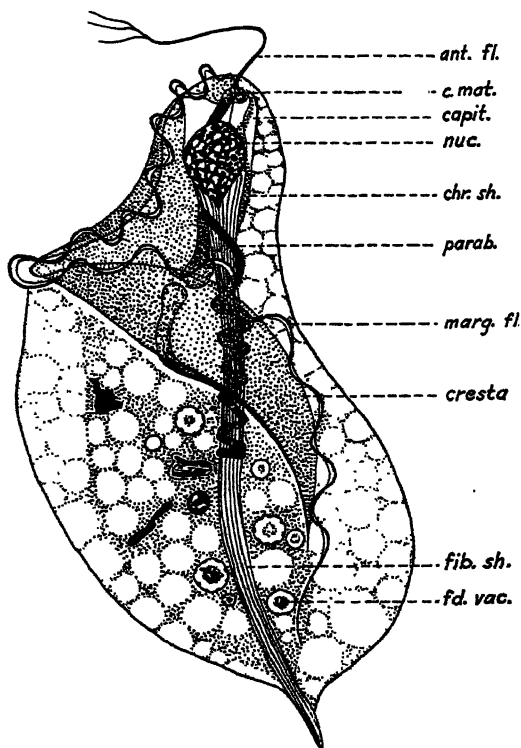
Fig. A. *Gigantomonas lighti* sp. nov. Drawn from the right side with aid of camera lucida, × 1015. *Ant. fl.*, anterior flagella; *capit.*, capitulum; *chr. sh.*, chromatic shield; *c, mat.*, centrosome matrix; *cresta*, cresta; *fd. vac.*, food vacuole; *fib. sh.*, fibrillar sheath of axostyle; *marg. fl.*, marginal flagellum of undulating membrane; *nuc.*, nucleus; *parab.*, parabasal body.

There are three anterior flagella which in life are adherent to one another throughout the greater part of their length, forming a whip (fig. A, *ant. fl.*). With dark-field illumination the individuality of the flagella can be distinguished near the tip of the whip. The cytoplasm is prolonged at the base of the flagella (pl. 8, fig. 1), forming

a tiny papilla. The whip behaves as though it possessed a tactile or exploratory function but it is practically valueless as an aid in locomotion. Fixatives, especially osmic iodide, cause the anterior flagella to separate from one another.

A fourth flagellum, which is much larger and more powerful than the anterior flagella, runs along the outer margin of the undulating membrane (fig. B, *marg. fl.*). The undulating membrane follows the
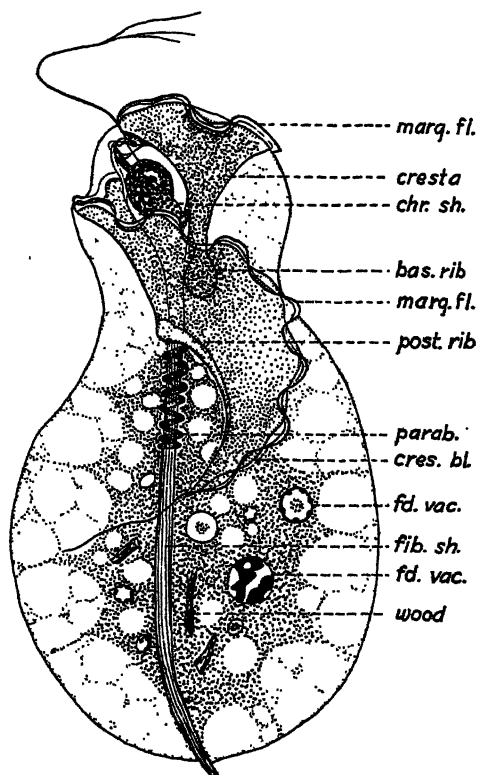
Fig. B. *Gigantomonas lighti* sp. nov. Drawn from the left side with aid of camera lucida, × 1015. *Bas. rib.*, Basal (antero-medial) reenforcement of cresta; *chr. sh.*, chromatic shield; *cres. bl.*, sculptured external (lateral) margin of cresta; *cresta.*, cresta; *fd. vac.*, food vacuole; *fib. sh.*, fibrillar sheath of axostyle; *marg. fl.*, marginal flagellum of undulating membrane; *parab.*, parabasal body; *post. rib.*, postero-medial reenforcement (rib) of cresta; *wood.*, wood.

external margin of the intracytoplasmic, undulatory cresta (pl. 8, fig. 1) from which it is easily torn by careless smearing and bulk fixation. The wave length of the undulations of the membrane is about one-sixth the wave length of the undulations of the cresta. Though the membrane is large and powerful, its width being about

double the width of its marginal flagellum, it seems to be of secondary importance in locomotion.

Although the cresta (fig. A. *cresta*) cannot be seen to advantage in life, it is visible and its movements can be easily followed. Its external (lateral) curved margin is in contact with the pellicle and is fused to the base of the undulating membrane except for a very short distance at its distal (posterior) end. It moves with varying
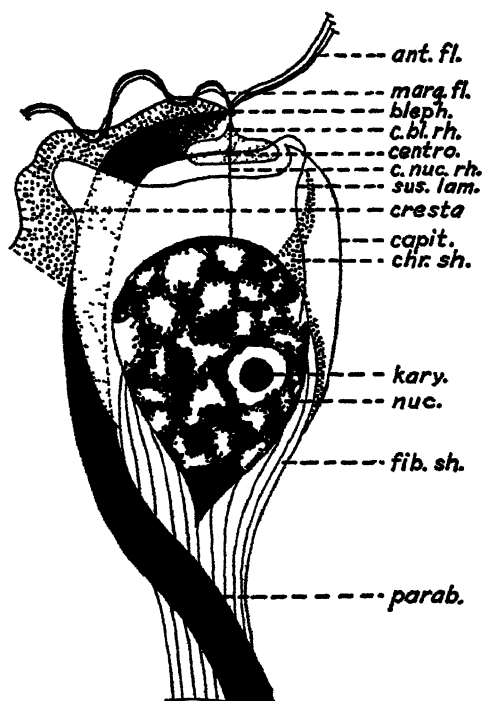
Fig. C. Anterior portion of neuromotor system of *Gigantomonas lighti* sp. nov., drawn with camera lucida, ×2500. *Ant. fl.*, anterior flagella; *bleph.*, blepharoplast; *capit.*, capitulum; *c. bl. rh.*, centriole-blepharoplast rhizoplast; *centro.*, centriole; *chr. sh.*, chromatic shield; *c. nuc. rh.*, centrosome-nucleus rhizoplast; *cresta*, cresta; *fib. sh.*, fibrillar sheath of axostyle; *kary.*, karyosome; *marg. fl.*, marginal flagellum of undulating membrane; *nuc.*, nucleus; *parab.*, parabasal body; *sus. lam.*, suspensorial lamella.

speeds and apparent power, its action scattering the vacuoles of the fluid portion of the cytoplasm throughout the body. The cresta is clavate in cross-section, thickening gradually from its external (lateral) margin toward its medial edges. Its antero-medial edge (base) is firmly fused to the axostyle. Following Dobell's (1909) orientation of *Trichomonas*, in which the chromatic basal rod or costa is regarded as dorsal, the line of fusion between cresta and axostyle may con-

veniently be adopted as indicating the dorsal surface of *Gigantomonas*.

The axostyle (fig. C, *capit.* and *fib. sh.*) is extremely refractile and can easily be traced in life from the region of the blepharoplast posteriorly to its tip at the other end of the cytosome. It is elastic and bends slightly under the repeated stress placed upon it by the undulating cresta. Anteriorly it lends support to the blepharoplast and nucleus; to its dorsal border the base of the cresta is fused and along this line of fusion the highly refringent parabasal body (fig. A, *parab.*) runs before coiling about the axostyle six to twelve times like a spring.

The nucleus (fig. C, *nuc*) is scarcely visible in life; its pyriform shape is made apparent by the refringent capitulum of the axostyle which almost completely encloses it.

The cytoplasm immediately surrounding the axostyle is of considerable density and frequently contains wood particles and numerous small vacuoles. Nearer the periphery of the cell the cytoplasm is quite fluid and the large liquid vacuoles which lie there are constantly being thrown violently about by the action of the cresta.

Along the posterior portion of the axostyle, numerous globules may be seen which after fixation exhibit a strong affinity for orange G (pl. 8, fig. 1). In organisms which have been observed for some time and in which degeneration is evident, the pellicle often pulls back along the axostyle leaving the globules adhering to it in a clump near its posterior end.

When the organism becomes moribund, the pellicle frequently ruptures and the fluid peripheral cytoplasm pours out, carrying with it the contained liquid vacuoles. As these pour from the break they come in contact with one another and coalesce so that fifteen or twenty such vacuoles may fuse to form a single drop. It was noted that these vacuoles possessed a slight affinity for neutral red.

Sphaerita, which is a frequent cytoplasmic parasite of *Gigantomonas*, may occur as highly refractile individual spores scattered throughout the cytoplasm (pl. 9, fig. 6) or clumped in spherical vacuoles.

Food taking has never been observed though numerous attempts have been made to discover the method used by this flagellate. As no cytostome is present it is probable that food is taken in directly through the pellicle as in many other polymastigote and hypermastigote flagellates of termites.

When Lugol's solution is applied to living material many organisms burst. In those which do not burst the cytoplasm and wood particles

stain so intensely that the organelles are obscured. Where the cytoplasm has flowed away, leaving the neuromotor system intact, both the cresta and axostyle are stained and the latter far more intensely, which may indicate, as Alexeieff (1929) shows for *Trichomonas augusta*, the presence of glycogen in the axostylar core.

Gigantomonas lighti moves slowly and with difficulty both in slide preparations and when observed directly through the unbroken wall of the gut. In a Locke's egg-white preparation it is practically helpless: through the wall of the gut it is seen to move rather less ineffectually among the struggling mass of flagellates. It is an interesting and surprising demonstration of the ineffectiveness of the trichomonad type of neuromotor system in a large protozoan even though carried to the highest development known in this great and wide-spread group.

OBSERVATIONS ON FIXED MATERIAL

SIZE

One hundred individuals were measured from fifteen slides which had been fixed in a variety of ways. The fixatives included the fluids of Schaudinn, Zenker, Zirkle, Champy, and osmic vapor. The measurements were made with an ocular micrometer and not more than seven or eight individuals were measured from any one slide. The largest organism measured $91\mu \times 50\mu$, the smallest $47\mu \times 40\mu$. The length varied from 47 to 91μ and the width from 21 to 58μ. The average size was $68\mu \times 38\mu$.

Dogiel found *Gigantomonas herculea* to be 60 to 75μ in length and 30 to 35μ in width. Thus *G. lighti* exhibits a much wider size variation than the type of the genus.

NEUROMOTOR SYSTEM

In *Gigantomonas lighti* the trichomonad type of neuromotor system reaches the highest degree of specialization known in this group. There are three anterior flagella (fig. C, *ant. fl.*) and a much larger flagellum (fig. C, *marg. fl.*) which runs along the external border of the undulating membrane. All the flagella arise from a single, coarse, chromatic granule, the blepharoplast (fig. C, *bleph.*). The cresta (fig. C, *cresta*) arises from the blepharoplast also and its external (lateral) border is delicately fused to the base of the undulating

membrane. The axostyle (fig. C, *capit.* and *fib. sh.*), the capitulum of which nearly encloses the pyriform nucleus, extends from the blepharoplast to and beyond the posterior limits of the body. Along its dorsal surface the base of the cresta is fused. In close association with and slightly posterior to the blepharoplast lies the centriole (fig. C, *centro.*). A stout, flattened fibril, the suspensorial lamella (fig. C, *sus. lam.*) extends ventrally from the centriole for a short distance before turning back posteriorly along the capitulum of the axostyle. The parabasal body (fig. C, *parab.*) has a double origin, one marginal filament arising from the blepharoplast, the other from the centriole. From its point of origin the parabasal body runs posteriorly to the right of the line of fusion between cresta and axostyle until at the posterior limit of the cresta's base it turns sharply about the axostyle in a leiotropic spiral.

DETAILED MORPHOLOGY OF THE NEUROMOTOR SYSTEM

The three anterior flagella, which average about 40μ in length, are all of equal size. The whip formed by their fusion is not uncommon among trichomonads, occurring in *Pentatrichomonas ardindelteili,* *Devescovina lemniscata* Kirby, and many others. The fourth or marginal flagellum, which borders the outer margin of the undulating membrane, continues at its distal end for a short distance as a free flagellum. It is much longer and stouter than the anterior flagella with which it shares a common origin at the blepharoplast. Both undulating membrane and cresta, as will be shown below, are probably derivatives of this marginal flagellum.

The blepharoplast is a single, intensely staining granule about 0.8μ in diameter. As the kinetic center of the cell it lies in close association with the mitotic center, the centrosome. Because of the duality of these two centers, a centroblepharoplast does not occur in the strict sense of the word. The centrosome consists of a strongly siderophile granule or centriole, lying in a matrix of much less chromophile material just posterior to the blepharoplast. A short rhizoplast (fig. C, *c. bl. rh.*) interconnects the blepharoplast and centriole, passing through the substance of the matrix. The centriole is about the size of the blepharoplast but the matrix, with a length of about 2.6μ and a width of about 1.5μ, is appreciably larger.

The suspensorial lamella, mentioned above, arises from the centriole, runs a short distance ventrally, then turns back posteriorly

over the right side of the capitulum of the axostyle before fading
from sight near the posterior limits of the nucleus. It is undoubtedly
homologous to the suspensorial lamella first described in *Devescovina
striata* var. *hawaiensis* by Janicki (1915) and later reported in
D. pruvoti by Duboscq and Grassé (1929). It may also be homologous
with the cytostomal fibril of *Trichomonas vaginalis* Donné described
by Hegner (1925) as well as the parablepharoplast bar and filament
of several other trichomonads (*Trichomonas barbouri* Kirby and *T.
cartagoensis* Kirby).

A well defined rhizoplast (fig. C, *c. nuc. rh.*), comparable to the
rhizoplast from the centroblepharoplast to the nuclear membrane of
so many flagellates, is found in the telophase. It is less easily demon-
strated during the interphase though occasionally it is found in prepa-
rations stained in Mallory's after Zenker's and in individuals fixed
in Zirkle's fluid.

The nucleus (fig. C, *nuc.*) is pyriform or top-shaped with the
pointed end directed posteriorly. It lies embedded in the spoon-shaped
capitulum of the axostyle. In size it varies from 10 to 12μ in length
and from 5.5 to 7.8μ in width. As in other Devescovininae the nucleus
is poor in chromatin, though the picture varies greatly with the
technique followed. In life the nucleus appears as a perfectly clear
vesicle and not at all refractile to light. After use of Lugol's solution
the karyosome can be faintly discerned. With fixatives lacking acetic
acid the cromatin lies in poorly defined patches on the inner face of the
delicate nuclear membrane. After Schaudinn's fluid the nuclear con-
tents so coagulate and contract that the chromatin appears massed in
the center of the nucleus with a clear space between it and the
nuclear membrane. Frequently upon fixation with Schaudinn's fluid
the chromatin comes to lie in two distinct masses, one the central mass
described above and another at the pointed posterior tip of the nucleus
which usually appears subtriangular in cross-section. Such a struc-
ture, undoubtedly a fixation artefact, is figured in both *Devescovina
hilli* and *D. pruvoti* by Duboscq and Grassé.

The karyosome (fig. C, *kary.*) which is about 2μ in diameter colors
with most nuclear stains but remains colorless after Feulgen's nucleal
reaction. Its position within the nucleus varies considerably but it is
most frequently found in the postero-ventral half of the nucleus.

Mitotic studies and the determination of chromosome number are
hampered by the peculiarities of the nucleus on fixation. Clear-cut
chromosomes have not been obtained without the aid of acid fixing

agents and after such treatment the chromosomes are found to lie imbedded in the surface of a dense plastin plug. Comparable difficulties were experienced by Cutler (1919) in his study of *Ditrichomonas termitis.*

The axostyle is a flexible, hyaline rod which runs from the region of the blepharoplast to project slightly beyond the posterior end of the body. It is exceedingly elastic and when bent quickly resumes its normal shape. This organelle is a compound structure consisting of the anterior portion or capitulum and the posterior fibrillar sheath. The capitulum (fig. C, *capit.*), clear and homogeneous, extends from near the blepharoplast posteriorly around the base of the nucleus and is prolonged as the core of the sheath. It completely surrounds the nucleus on its dorsal, ventral, and left faces. Only on the right anterior and anterior faces the nucleus is not so protected.

Surrounding the core and partly surrounding the nucleus is the fibrillar sheath (pl. 9, fig. 11). This is composed of many delicate chromophobic fibrils laid down around the core in fine lamellae (fig. C, *fib. sh.*). Such an arrangement gives to the posterior portion of the axostyle its characteristic striated appearance. On the left side the fibrillar sheath extends nearly to the anterior limits of the nucleus but falls away gradually both dorsally and ventrally until on the right side the fibrils reach only a short distance ahead of the posterior tip of the nucleus.

From the base of the nucleus to the posterior tip of the body, the axostyle, though nearly cylindrical, tapers gradually and, as it leaves the body, comes quickly to a point. On its course through the body the diameter of the axostyle decreases from about 8μ at the base of the nucleus to about 5μ at the point where it leaves the body.

Alexeieff (1924) has suggested that the axostyle is probably homologous with a flagellum, listing as common characteristics stainability, contractility, and origin from the blepharoplast. Though the axostyles of *Giardia, Octomitus,* and other Diplozoa are intracytoplasmic portions of the posterior flagella and retain evidences of their origin, the axostyle of the Devescovininae is so highly modified that redifferentiation at cell division, morphological position, and a common origin from the blepharoplast are the only characteristics which it shares with flagella. Alexeieff also maintains that the axostyle serves primarily as a rudder. Such may be the case in organisms possessing a contractile axostyle but in *Gigantomonas* the axostyle functions primarily as a cytoendoskeleton and, as Dogiel (1916) notes, forms an elastic base against which the cresta beats.

The cresta may be compared in shape to the sharply curved blade of a knife. The base or antero-medial edge is firmly fused to the axostyle. The line of fusion, which extends from the blepharoplast just beyond the posterior tip of the nucleus, determines the dorsal side of the organism. The external (lateral) edge of the cresta is fused at the pellicle with the base of the undulating membrane. Short siderophile lines which lie parallel to one another and perpendicular to the external edge of the cresta delicately sculpture its margin (pl. 9, fig. 7). From its external edge toward its medial edges the cresta gradually increases in thickness. The antero-medial edge or base averages about 23μ in length, the external (lateral) edge about 70μ, and the postero-medial edge about 50μ. Though the cresta increases gradually in thickness from the external margin, the base is further strengthened along the line of fusion with the axostyle by an additional thickening which terminates abruptly in an ovate enlargement near the posterior limit of the base (pl. 9, fig. 7). Beyond this ovate termination of the basal thickening an unreinforced portion of the base continues along the line of fusion for a few microns. The postero-medial edge of the cresta is likewise reinforced by a rib-like enlargement which extends from near the point of the blade back almost to the ovate portion of the basal thickening where it terminates abruptly in a rounded end (pl. 9, fig. 7). A narrow, unreinforced space occurs between the basal enlargement and the rounded proximal end of the postero-medial rib which serves as a hinge when the cresta beats.

The cresta is here no passive support for the long posterior flagellum as in most other described Devescovininae, but an actively undulating, intracytoplasmic organelle which is the most important factor in locomotion as it forces the undulating membrane by its powerful sweep against the semi-fluid contents of the gut. Its wave-like movement gains support from the axostyle to which the base of the cresta is fused and as the cresta forces the organism through the struggling mass of flagellates which fill the gut, the axostyle is repeatedly bent under the constant strain of its beat.

In fixed material the cresta is frequently seen tightly wound about the anterior portion of the axostyle. Such a position has never been noted in life and seems to result from sudden contraction upon fixation.

The chromatic shield (fig. B, *chr. sh.*; pl. 9, fig. 8) is an orderly arrangement of chromatic spherules which lie against the clear homogeneous substance of the capitulum. The shield-like arrangement of the spherules seems quite constant and their numbers do not vary

appreciably. They stain strongly with basic stains but neither Lugol's solution nor Feulgen's will color them. It is probable that here in *Gigantomonas* the chromatic spherules, which in so many trichomonads are found along the axostyle, beneath the costa (paracostal granules of Grassé), and scattered about the anterior end (frontal granules of Grassé), are collected in a sharply delimited portion of the cytosome.

In *Gigantomonas lighti* the parabasal body (fig. A, *parab.*, and fig. C, *parab.*) reaches the greatest development known in any of the polymastigote flagellates. Though the parabasal body of *Gigantomonas herculea* was not seen by Dogiel it is probable that it reaches a size comparable to that of *G. lighti*. In shape it is nearly cylindrical and in length it varies considerably according to the individual. The longest parabasal found untwisted from the axostyle measured 102μ in length. It is possible to estimate roughly the length of the parabasal as the length of the base of the cresta plus the product of the circumference of the axostyle times the number of turns which the parabasal makes about it. Such estimates show that many of these organelles must reach a length well over 200μ. The diameter of the parabasal varies slightly with the fixative used as osmic preparations tend to make it appear swollen while Schaudinn's fluid seems to contract it. After Champy's fluid the diameter averages about 2.5μ.

It is interesting to note that in those trichomonads in which there is a centroblepharoplast and the parabasal has been studied, this organelle seems to arise from the centroblepharoplast by a single marginal filament or parabasal thread as Janicki (1915) originally called it. In *Gigantomonas lighti,* possessing as it does the blepharoplast and centrosome as separate and discrete bodies, however closely associated they may be, the parabasal has a double origin. One marginal filament, the superior marginal filament, arises from the blepharoplast and apparently extends throughout the length of the body. A second marginal filament, the inferior marginal filament, arises from the centriole and can be traced back for only a short distance before it fades from view (fig. C, *parab.*).

The parabasal body, running from its origin posteriorly to the right of the line of fusion between the axostyle and the base of the cresta, turns about the axostyle in a leiotropic spiral at the posterior extremity of the cresta's base. In post-mitotic individuals which are not yet completely reorganized, the parabasal is rather loosely wound (pl. 9, fig. 13) but later becomes as tightly wound as a spiral spring.

It has been commonly held that acetic acid dissolves or greatly alters the parabasal body, a concept further heightened by the fact that many workers, particularly the earlier ones, using the fluid of Schaudinn and Heidenhain's haematoxylin failed to note the parabasal at all. In *Gigantomonas lighti* the parabasal body is not dissolved by acetic fixatives though it appears to be somewhat altered. When material fixed in Schaudinn's fluid is stained with Heidenhain's haematoxylin the parabasal colors only in overstained individuals (pl. 9, fig. 9). In organisms that have been destained sufficiently to study other cell structures the parabasal is so completely destained that it can scarcely be traced save by its displacement of chromatic granules in the cytoplasm. If Delafield's haematoxylin is used to stain material fixed in the same fashion the parabasal colors nicely (pl. 9, fig. 10). It is after such fixation and staining that the parabasal appears as a strongly chromophile matrix containing two approximately parallel rows of chromophobic vesicles. Near the pointed distal end of the parabasal the rows of vesicles lose their regular arrangement and are embedded near the surface of and entirely around the matrix. After fixation in the fluids of Zenker, Champy, Zirkle, and osmic vapor followed by either Heidenhain's or Delafield's haematoxylin the vesicular structures of the parabasal could not be detected. After osmic fixation particularly, the parabasal seems to be composed of the marginal filaments and numerous fine, intensely staining granules dispersed throughout a clear ground substance. At times the surface of the parabasal appears to be a definite membrane.

The investigations of Grassé (1926) and several papers of Duboscq and Grassé have added much to our knowledge of the nature of the parabasal body. They have advanced three interesting hypotheses which merit attention. First, that a homology exists between the idiosome of the spermatid and the parabasal of polymastigote and hypermastigote flagellates and that the parabasal is therefore comparable to the Golgi material of metazoan cells. Secondly, that the parabasal (kinetonucleus) of *Herpetomonas* and the parabasal of Bodonidae differ fundamentally from the parabasals of polymastigote and hypermastigote flagellates so that two distinct types of parabasal bodies occur among the Zoomastigoda. Lastly, that the parabasal of polymastigote and hypermastigote flagellates possesses a secretory function, a hypothesis suggested by the structure and supposed Golgi affinities of the parabasal, was advanced by Grassé in his wholly admirable paper of 1926.

King (1927) has summarized the evidence supporting these views as follows:

1. Acetic acid destroys or greatly alters both Golgi material and the parabasal body. That the parabasal of *Herpetomonas* and *Bodo* persists after such treatment is due to a higher proportion of protein to lipoid matter than is found in the parabasal of higher forms. Similar differences of resistance are found in the Golgi bodies of metazoan cells.

2. Both can be demonstrated after mitochondrial fixatives by iron haematoxylin and can be impregnated by osmic and silver methods.

3. In all cases that have been thoroughly studied, the parabasal divides at cell division and is thus self-perpetuating as is Golgi material.

4. The parabasal is always associated with the blepharoplast or centroblepharoplast, comparable to the arrangement of the Golgi material of metazoan cells about the centrosome.

5. There is some evidence that the parabasal, like Golgi material, is secretory in nature.

Kirby (1931*b*) has thoroughly criticized and evaluated the above evidence. His statements which coincide with the results obtained with *Gigantomonas* are listed below with certain additional evidence from other workers:

1. Causey, Hall, and Brown working on *Peranema, Chilomonas, Chromulina, Euglena,* and *Dinenympha* have found dispersed dictyosomes of a Golgi-like material. Brown (MSS) more recently has found comparable structures in *Trichonympha campanula.*

2. The parabasal body is not dissolved by acetic acid or mercuric chloride because it will stain intensely with Delafield's haematoxylin after such fixation. In *Gigantomonas* it appears to be somewhat altered by such treatment.

3. It is preserved by mitochondrial and Golgi techniques but also remains after methods which destroy Golgi material.

4. Duboscq and Grassé found a chromophile and chromophobe structure which could be demonstrated only after considerable destaining. The chromophilic portion is perhaps comparable to the marginal filaments of Janicki. The clear chromophobic vesicles are supposedly secretory products, though secretion has never been satisfactorily demonstrated. Other fixatives and stains give markedly different results; at times the pictures given by one technique may be exactly

reversed by another. Thus the portions which appear chromophilic with one basic stain may appear chromophobic with another.

5. The parabasal may divide in *Bodo, Proteromonas,* and the Trypanosomidae but the parabasal of these organisms may not be homologous with the parabasal of trichomonad flagellates. The parabasal (kineto-nucleus) of *Herpetomonas* stains with Feulgen's nucleal reaction but no parabasal of any trichononad has ever been reported as staining by this method.

Janicki (1915) was unable to determine whether the parabasal of *Devescovina striata* divided, though it appeared to be possible. His cautious statement has been overlooked and a positive statement of division of the parabasal has commonly been attributed to his pen. Wenrich (1921) believed that at least one new parabasal was redifferentiated in *Trichomonas muris.* Alexeieff (1924) states that during division of *Trichomonas augusta* the old parasome (parabasal) is resorbed and that two new parabasals are formed at the expense of the mitochondria. The redifferentiation of the parabasal from mitochondria cannot as yet be accepted but that new parabasals are redifferentiated at cell division has been confirmed by Kirby (1930*b*; 1931*a* and *b*) and the writer.

The rapidity with which the parabasal bodies are redifferentiated during the early prophase of division coupled with the occasional variations (split ends, double proximal portions, and even double parabasals) which occur in interphase individuals, was undoubtedly responsible for the earlier, rather general belief in the division of trichomonad parabasals during mitosis.

To summarize briefly, the Golgi affinities of the parabasal bodies of trichomonad flagellates seem remote because these structures are not destroyed by fixatives which supposedly destroy Golgi material, they are not self-perpetuating, and a secretory function cannot be regarded as definitely proved.

The appearance of the cytoplasm in life has been described above. In fixed material the living condition is most nearly simulated by use of the fluid of Zirkle for fixation.

The cytoplasm of *Gigantomonas lighti* is frequently parasitized by *Sphaerita,* a fungus usually placed in the Chytridiales. The ovoid spores generally occur in spherical clusters though occasionally they may be scattered through the whole cytoplasm (pl. 9, fig. 6). In a few individuals the cytoplasm was crowded with a peculiar crescentic organism which shows a strong affinity for basic stains (pl. 9, fig. 5).

In one or two instances this organism and the spores of *Sphaerita* were both found in the same individual. It may be that this second organism is a stage in the life-cycle of Sphaerita though no method was found to ascertain the truth of such an assumption.

Anatomical abnormalities and peculiarities are not unknown in *Gigantomonas lighti,* nor is it surprising that such variations should occur when one considers the extreme complexity of this flagellate and the complete redifferentiation of organelles which occurs at each division. Most common of these variations are those of the parabasal body, mentioned above. One individual was found in which all organelles save the distal end of the axostyle were doubled (pl. 30, fig. 12). It is unlikely that this individual would have reproduced forms like itself for it apparently arose by a chance fusion of the growing daughter axostyles at the preceding mitosis. No reversal of symmetry occurred in either daughter neuromotor system. The presence of such abnormalities demonstrates the necessity for studying many individuals before drawing conclusions as to the redifferentiation of organelles.

DIVISION

Division in *Gigantomonas lighti* is a most complex process involving the dedifferentiation and resorption or extrusion of all neuromotor organelles followed by the redifferentiation of an entirely new neuromotor system. Because so many processes are going on at once and because the organization of *Gigantomonas* is so complex, it seems better to give in brief outline the whole process of division, correlating the changes in various organelles, and then to follow in a detailed fashion the fate of each organelle.

Dividing individuals may be found in the gut four to six days after ecdysis. Termites sacrificed before or after this period are usually valueless for studies of division. Mitotic flares may be artificially induced by partial defaunation with oxygen pressure but frequent culling of the colony for newly moulted animals proved so satisfactory that the oxygen method was used but twice.

The first sign of approaching mitosis is the division of the centriole, followed soon by division of the blepharoplast. When the blepharoplast divides there is an unequal distribution of flagella between the daughter blepharoplasts. New anterior flagella grow out very quickly and for a time each blepharoplast supports more than the normal

complement of flagella. A paradesmose forms between the daughter centrioles (pl. 8, fig. 2). The chromatic shield breaks up as the capitulum is gradually resorbed and the spherules lie scattered about near the nucleus. Next the cresta and parabasal body lose connection with the neuromotor system and lie free in the cytoplasm. The cresta disappears rapidly but the parabasal body may be observed until the late prophase before it at last becomes completely resorbed (pl. 8, figs. 2, 3). During the prophase, in addition to the replacement of the anterior flagella, one new marginal flagellum appears, which grows slowly, reaching nearly full development in the late telophase. The new parabasal bodies appear in the prophase (pl. 8, fig. 2); they grow rapidly, reaching almost complete development before the metaphase (pl. 8, figs. 3, 4). The fibrillar sheath of the axostyle breaks away from the nucleus at the approach of the metaphase (pl. 8, fig. 4) and two new axostyles are redifferentiated during the telophase (pl. 9, fig. 14). The cresta is also redifferentiated in the telophase (pl. 9, fig. 14) though it does not complete its development until affer separation of the daughter cells. The old marginal flagellum is replaced just before plasmotomy. Thus the parts of the two new neuromotor systems have been redifferentiated before the daughters separate, though reorganization is not complete until some time after plasmotomy.

DETAILED OBSERVATIONS ON MITOSIS

At the approach of mitosis the centrosome divides and two chromatic granules, the centrioles, may be noted in the matrix which surrounds the division center of the cell. The daughter centrioles are interconnected by a delicate, strongly chromophilic rhizoplast which persists throughout division. A rhizoplast also runs from each daughter centriole to the blepharoplast. The inferior marginal filament of the parabasal body retains for a time a connection with the more dorsal of the daughter centrioles while the suspensorial lamella remains attached to the other. As the daughter centrosomes separate more widely, numerous fibrillae, less chromophilic than the interconnecting rhizoplast, appear parallel to and around it forming the paradesmose proper (pl. 8, fig. 2). The centrioles lie near the tips of the paradesmose and embedded in its substance. Thus the paradesmose of *Gigantomonas lighti* corresponds closely to the fibrillar paradesmose or extra-nuclear spindle of *Devescovina* which Janicki (1915) described.

In the meantime, the blepharoplast has divided with an unequal distribution of flagella, one daughter taking the marginal flagellum and one or two anterior flagella, the other daughter taking the rest. New anterior flagella are soon redifferentiated, so for a time four anterior flagella are found at one blepharoplast and five at the other. Before the anaphase the old anterior flagella have disappeared and the normal complement of three anterior flagella is found arising from each blepharoplast. Throughout mitosis a rhizoplast connects each blepharoplast with its corresponding centriole, a connection that is maintained throughout life.

The cresta and parabasal body lose connection with the neuromotor system early in the prophase and lie free in the cytoplasm until they are resorbed (pl. 8, figs. 2, 3). The cresta disappears rapidly but the parabasal body is more persistent (pl. 8, figs. 2, 3), bits of it remaining distinguishable until the chromosomes appear and long after the new parabasals are well developed.

The capitulum, unlike other organelles, does not break away from the neuromotor system but is resorbed *in situ* and the spherules of the chromatic shield which adhere to its surface lose their regular arrangement and lie scattered in the cytoplasm about the nucleus and the tips of the paradesmose. The spherules gradually disappear during division. Though the capitulum is resorbed early in the prophase, the fibrillar sheath of the axostyle is far more persistent and does not lose connection with the nucleus until the approach of the metaphase (pl. 8, fig. 4). In addition to being the last organelle of the old neuromotor system, save the marginal flagellum, to become nonfunctional, the fibrillar sheath is also the slowest to show any sign of resorption and persists in recognizable form into the late telophase (pl. 9, fig. 14).

Thus far we have been concerned chiefly with the process of dedifferentiation and have seen it to be practically complete by the metaphase. The fibrillar sheath and the marginal flagellum are the sole organelles which remain for later consideration. Changes which mark the beginning of redifferentiation of new neuromotor organelles will now be considered in detail.

Following division of the centrosome and development of the paradesmose, changes preparatory to the formation of chromosomes begin in the nucleus. The karyosome disappears and the chromatin becomes arranged in irregular masses. Anteriorly the nuclear membrane

becomes fused with the paradesmose and as the paradesmose elongates the nuclear membrane is drawn out at its tips (pl. 8, fig. 2).

The appearance of the nucleus varies considerably with the fixative used. After Schaudinn's, the chromosomes, which emerge from the irregular masses mentioned above, appear block-like and lie embedded upon the surface of a dense plastin plug which is about half the diameter of the nucleus and lies in the center of it. After Zirkle's fluid the chromosomes seem more granular and less clean-cut. An intranuclear spindle is present which, following Schaudinn's fixation, seems to form by coagulation the dense plastin plug (pl. 8, fig. 4; pl. 9, fig. 15).

The chromosomes in the prophase appear to split longitudinally and remain closely interconnected by several chromatin strands. There are five pairs of chromosomes, of which one pair is always considerably smaller than the rest. Each member of the other four pairs of chromosomes appears to be about twice as long as wide.

As the chromosomes are directed toward the equatorial plate by the spindle fibers they pull apart (pl. 8, fig. 4) and begin their migration toward the poles of the spindle. The small pair, which migrates more quickly than the other chromosomes, reaches the poles while the others are still in the anaphase position (pl. 9, fig. 15).

In the telophase the chromosomes first become clustered at the poles of the intranuclear spindle which lie at the tips of the paradesmose. They then become less dense and finally give rise to the scattered granules of the interphase nucleus (pl. 9, fig. 14). As the chromatin granules become more dispersed a small ovoid body, which stains intensely, appears at one side of the chromatin mass. A halo forms about it and thus the karyosome is replaced. It may be worthy of note that the karyosome, which stains intensely with haematoxylin and other basic stains, fails to react at all to Feulgen's nucleal reaction.

The new parabasal bodies become visible in the early prophase and the marginal filaments, which grow out first, form at this time nearly the whole substance of these organelles. As in the interphase, one marginal filament originates at the blepharoplast and the other at the centriole. The parabasal bodies are redifferentiated more quickly than any other organelle and attain nearly their full size during the prophase (pl. 8, figs. 3, 4). They remain twisted about in the cytoplasm, growing slightly larger all the time, until, after plasmotomy, they become curled about the axostyle (pl. 9, fig. 13).

Though the anterior flagella are soon redifferentiated, developing from the daughter blepharoplasts at the same time, such is not the case with the marginal flagella. One new marginal flagellum can first be discovered in the late prophase and grows slowly from that time until reorganization is completed after plasmotomy (pl. 8, fig. 4: pl. 9, figs. 14, 15). At no time before the daughter cells separate does it ever quite equal the old marginal flagellum in either length or diameter. Because of this size difference it is not difficult to distinguish between the old and the new.

The old marginal flagellum remains functional longer than any organelle of the old neuromotor system. Not until the late telophase does it finally become detached from the blepharoplast. Even then it appears to adhere for some time to the surface of the cytosome and may remain functional until the second new marginal flagellum reaches considerable size. Whether it is finally discarded or resorbed could not be determined. The second new marginal flagellum seems to grow out along the old flagellum and enlarges more rapidly than the first for at plasmotomy, which soon occurs, both new marginal flagella are of about equal size.

The new axostyles appear in the early telophase and form in close association with the nuclear membrane. The capitulum, which is connected to the centriole by the suspensorial lamella, forms first, partly surrounding the nucleus, and the core of the axostyle grows out from it beyond the base of the nucleus. While the core is yet a short, stubby rod, numerous fibrils, destined to form the fibrillar sheath, arise in the cytoplasm and are laid down around it (pl. 9, fig. 14). Growth continues until after plasmotomy and probably throughout life. When the axostyle first appears a stout chromophilic rhizoplast may be seen running from the nuclear membrane to the centriole which is probably the centriole-nuclear membrane rhizoplast that is seen so infrequently in adult organisms.

Even after the new axostyles are well developed the old axostyle may still be seen lying free in the cytoplasm of the cell. Though other organelles are soon resorbed the axostyle seems more resistant and in many late telophases it does not appear to be particularly changed. It is possible that the old axostyle is finally extruded from the body rather than resorbed. If it is resorbed the process must proceed rapidly once it has begun, because no trace of the old axostyle has ever been noted in newly divided forms.

The cresta is the last organelle to reach complete redifferentiation. In the telophase it appears as a delicate membrane growing back into the cytosome from the base of the each undulating membrane. Its basal enlargement and its strengthening postero-medial rib appear only after plasmotomy and as the axostyle is only partly developed there is no fusion between it and the base of the cresta. Thus for a time the cresta appears to be only an intracytoplasmic, undulating membrane and its curves tend to follow the undulations of the external membrane. It is not until after separation of the daughter cells that the cresta completes its growth and acquires the sturdy supporting ribs which give to it the ability to exert the strength and power which so well characterize its action in life.

Before plasmotomy occurs the paradesmose becomes greatly lengthened and bent. As no trace of it has ever been found in newly divided forms it probably disappears rapidly after plasmotomy.

When plasmotomy occurs the protoplasmic mass of the organism is divided equally between the two neuromotor systems which soon become reorganized as adult individuals (pl. 9, fig. 13).

DISCUSSION

SYSTEMATIC POSITION AND COMPARISONS

As Kirby (1931a) has suggested, trichomonad flagellates fall naturally into three subfamilies, the Polymastiginae with *Polymastix, Monocercomonas, Eutrichomastix,* and others; the Trichomonadinae, with *Trichomonas* and closely allied genera; and the Devescovinnae (Devescovinidae Poche 1913) with *Devescovina, Gigantomonas, Foaina, Parajoenia, Paradevescovina, Metadevescovina,* and *Janickiella.*

The Devescovininae are characterized by three anterior flagella and a much larger posteriorly directed flagellum which may or may not adhere to the pellicle forming an undulating membrane. Beneath and at the base of the posteriorly directed flagellum lies the cresta which may be subtriangular or rod-like. The cresta is usually small and appears to support and, perhaps to some extent, direct the posterior flagellum. In *Gigantomonas* it becomes a powerful undulatory organelle to which the base of the posteriorly directed flagellum is fused, forming a large, undulating membrane. The cresta has appeared in literature under the following names: chromatic basis, chromatic

basal rod, côte, costa, costule, cresta, parabasal body, and Schlepp-geisselscheide. The term *cresta* originated by Grassi (1917), has been retained by Kirby (1931*a*) because it has not been used for the homologous but functionally different costa or chromatic basal rod of *Trichomonas* and closely allied genera.

The axostyle of the *Devescovininae* is well developed and in many cases possesses a homogeneous core and a fibrillar sheath.

The parabasal body is prominent in all members of the group and commonly makes from one to a dozen turns about the axostyle.

Gigantomonas lighti sp. nov. is the most complex of all described members of this well defined subfamily, and in fact is the most highly organized of all trichomonad flagellates. In it the neuromotor organelles, which become progressively enlarged and modified as one traces the phylogeny of the group from *Eutrichomastix* through *Devescovina* and *Janickiella* to *Gigantomonas*, attain a development unknown in other trichomonads.

Whether Dogiel's *Gigantomonas herculea*, type species of this genus described from *Hodotermes mossambicus* of British East Africa, attains the degree of specialization found in *G. lighti* cannot be easily ascertained. Professor Dogiel has been unable to supply the writer with material for comparison and it has been only with great difficulty that *G. lighti* and *G. pulchra* have been ascribed to that genus.

That Dogiel's description and figures are incomplete may be explained by the technique used by him while collecting in the field. His methods, which are not described in the English abstract of his paper, are given in the Russian text. Some smears were made and fixed in Schaudinn's fluid (cold ?) but apparently most of his material was prepared by dropping the gut of the termite into test tubes containing a fixing agent. For fixatives the fluids of Gilson, Schaudinn, and Flemming were used. The organisms were recovered from a mixture of wood particles and Protozoa found on the bottom of the test tube and from sections of the gut. Apparently no living material was observed. Borax carmine, Delafield's haematoxylin, Giemsa, safranin and light green, and Heidenhain's iron haematoxylin (which proved best for neuromotor organelles) were used for stains.

The writer has duplicated the methods followed by Dogiel and found them wanting as a basis for any careful morphological study of so delicate an organism as *Gigantomonas*. The artefact most frequently induced by such treatment is the complete detachment of

the posterior or marginal flagellum and undulating membrane from the outer margin of the cresta. This led Dogiel to confuse the marginal flagellum with the anterior flagella and the undulating membrane with the cresta. Other errors of a similar nature, coupled with an insufficient description, made the comparison attempted below difficult.

In addition to *Gigantomonas herculea,* Dogiel described from the gut of *Hodotermes mossambicus* a similar flagellate, *Myxomonas polymorpha,* which is characterized by permanent amoeboid form, absence of cytostome, and absence of anterior flagella. The six types of *Myxomonas* described are listed below.

Type A. Possesses an axostyle, undulating membrane (cresta), and nucleus. Flagella may have been present but their presence could not be definitely determined. Food particles rarely present.

Type B. No axostyle. All one type in a single termite. Food particles rare.

Type C. Motor organelles much reduced. Some precystic (?) forms.

Type D. Numerous food particles in cytoplasm. Dividing forms with fibrillar paradesmose and numerous chromosomes (about 100).

Two other types, E and F, are described, the first as a binucleate type C and the latter, type F, as the product of a peculiar budding from type E (probably degenerate).

Types A and B, because of the scarcity of food particles in the cytoplasm, were suggested as stages preparatory to sexual reproduction.

A close comparison of the various *Myxomonas* types with division stages of *Gigantomonas lighti* reveals a striking similarity of form and structure. That *Myxomonas* is synonymous with *Gigantomonas* seems probable for the following reasons. The termite host was not found to be generally infected with all types, but rather a single termite tends to harbor a single type. The condition found in natural mitotic flares is quite comparable, for all dividing individuals from the gut of any one termite tend to be in about the same stage of mitosis. The prophase tends to be long and prophase figures, comparable to types A and B, are commonly found without later stages appearing on the slide. "Pseudopodia," which characterize *Myxomonas,* are frequently produced when dividing forms are smeared upon a slide; at times the organism may stretch ten times its normal length without breaking. Moreover, loss of flagella was not determined and could not be by

the methods and technique employed. Reduction of neuromotor organelles was no greater than normally occurs at division. Lastly, no division stages of *Gigantomonas* were noted, a fact scarcely to be explained in so general a mitotic flare as reported for types D and E.

Reichenow (1928, p. 741) suggests that *Endamoeba disparata* Kirby and other xylophagus amoebae from Central American Termitidae may have arisen from the parasitic flagellates of termites and that *Myxomonas* is itself a transition stage between flagellate and amoeba. Kirby (1926) has shown the probable relationships between the *Endamoeba* species infecting Termitidae and *E. blattae* of the cockroach and suggests that "the line of development must lead to the ancestors common both to termites and the cockroach." There is no evidence to show that the *Myxomonas* types described by Dogiel are other than division stages and degenerating individuals of *Gigantomonas*. Moreover, the origin of characteristic *Endamoeba* species from the highly differentiated flagellates of termites is fantastic to say the least.

Kudo (1931, pp. 160, 161) has also preserved the genus *Myxomonas* rather than placing it in synonomy.

Three species of Devescovininae are known in which the large posteriorly directed flagellum is fused to the external margin of the cresta at the pellicle, forming an undulating membrane. Of these, *Gigantomonas herculea* Dogiel 1916, was the first to be described and thus has priority. *Macrotrichomonas pulchra* Grassi 1917 and *Gigantomonas lighti* sp. nov. are the other known species. They differ from one another only in details of morphology and are found in different species of termite host. After a careful study of *G. lighti* and the papers of both Dogiel and Grassi, the writer believes that, as Grassi himself suggested, the genus *Macrotrichomonas* is synonomous with *Gigantomonas* Dogiel 1916.

A TABULAR COMPARISON OF THE SPECIES OF GIGANTOMONAS

Size	*G. herculea* 60–75μ x 30–35μ	*G. lighti* 47–91μ x 21–58μ	*G. pulchra* About 57μ x 36μ from magnification given by Grassi
Anterior flagella	Three arising from a papilla	Three arising from a papilla	Probably three arising from a papilla
Undulating membrane	Bordered by a heavy marginal flagellum, pulled free in preparations from external border of cresta	Bordered by a heavy marginal flagellum. Base fused to external border of cresta	Bordered by a marginal flagellum, less well developed than in other two species. Base fused to external border of cresta
Cresta	Anterior and posterior medial borders reinforced by gradual thickening from antero-lateral margin. Undulatory. External and postero-medial edges figured relatively longer than in *G. lighti*	Thickened from external margin toward both medial edges which are additionally reinforced by rib-like enlargements. Undulatory. External edge............70μ Antero-medial edge 23μ Postero-medial edge 50μ	Antero-medial edge only reinforced. Probably undulatory. Smaller than in other two species. External edge............33μ Antero-medial edge 15μ Postero-medial edge 22μ
Axostyle	1. Capitulum part-figured, not described. 2. Posterior to nucleus axostyle consists of chromophile core and fibrillar sheath	1. Capitulum well developed, nearly encloses nucleus. 2. Capitulum prolonged posterior to nucleus as slightly chromophile core surrounded by chromophobe fibrillar sheath	1. Capitulum less well developed than *G. lighti*. 2. Structure not described. No figures showing posterior portion. Probably has fibrillar sheath as in other two species
Nucleus	Ovoid Karyosome present	Pyriform, pointed end directed posteriorly Karyosome usually in left ventral portion of nucleus	Ovoid. Karyosome present
Chromatic shield	Neither figured nor described	Orderly arrangement of chromatic spherules adherent to left face of capitulum	Neither figured nor described

Size	*G. herculea* 60–75µ x 30–35µ	*G. lighti* 47–91µ x 21–58µ	*G. pulchra* About 57µ x 36µ from magnification given by Grassi
Parabasal body	None described. Probably present	Well developed. Two marginal filaments. Makes six to twelve turns about fibrillar sheath of axostyle	Well developed. Makes four to a dozen turns about axostyle. Marginal filaments neither figured nor described
Blepharoplast	Neither figured nor described	Single intensely staining granule from which flagella, cresta, and one marginal filament of parabasal arise	Figured as from one to four small chromophile granules variously arranged beneath papilla
Centrosome	Neither figured nor described	During interphase, a single chromophile granule (centriole) lying in supporting matrix just posterior to blepharoplast to which it is connected by a rhizoplast. One marginal filament of parabasal arises dorsally and suspensorial lamella arises ventrally from the centriole	Neither figured nor described
Pellicle	Thick?	Very delicate	Probably delicate
Pseudopodia	Described but probably artefacts induced by smearing	Never seen in living material. Commonly present in fixed smears	Not described
Chromidium	Extra-nuclear karyosomes thrown off by nucleus forming an ovoid chromophilic structure similar in appearance to the chromidium of rhizopods and some gregarines. Presence and described origin questionable. Possibly poorly preserved parabasals	No comparable structure	No comparable structure

Gigantomonas Dogiel

Gigantomonas Dogiel 1916, pp. 1–14, pl. 1, figs. 6–12.
Myxomonas Dogiel 1916, pp. 15–33, pls. 1–4, figs. 13–44.
Macrotrichomonas Grassi 1917, pp. 376–378, pl. 9, figs. 1–12.

Diagnosis.—Large devescovinid flagellates (described species 47–91μ long by 21–58μ wide). Three fine anterior flagella, one large posteriorly directed flagellum which follows outer margin of undulating membrane. Base of undulating membrane fused to external (lateral) margin of cresta. Cresta of dense granular cytoplasm, intracytoplasmic and undulatory. Antero-medial edge of cresta fused firmly to axostyle. Nucleus ovoid or pyriform, with karyosome, supported by capitulum of axostyle which is prolonged posteriorly as the axostylar core. Core surrounded by fibrillar sheath. Parabasal body single, cylindrical where noted, makes four to twelve turns about fibrillar sheath of axostyle.

Type species.—*Gigantomonas herculea* Dogiel 1916 from the intestine of *Hodotermes mossambicus* Hagen, a termite from British East Africa.

Gigantomonas herculea Dogiel 1916

Diagnosis.—Shape pyriform. Length 60 to 75μ, width 30 to 35μ. Three fine anterior flagella and a larger posteriorly directed flagellum which follows the external border of a long, well developed undulating membrane. Cresta thickened from external border toward medial edges, postero-medial edge without rib-like reenforcement, antero-medial edge fused to capitulum. Axostyle, posterior to nucleus, has chromophilic core surrounded by a fibrillar sheath. Nucleus, supported by axostyle, ovoid and with karyosome. Parabasal body undescribed but undoubtedly present. Pellicle thick.

Host.—*Hodotermes mossambicus* Hagen, a termite from British East Africa.

Gigantomonas pulchra (Grassi 1917)

Macrotrichomonas pulchra Grassi 1917, pp. 376–378, pl. 9, figs. 1–12.

Diagnosis.—Shape pyriform. Length about 57μ, width about 37μ. Three anterior flagella and a fourth larger flagellum fused to external border of cresta forming an undulating membrane about the length of the body or shorter. Cresta small, antero-medial edge thickened and fused to capitulum, postero-medial edge without thickening or rib. Nucleus ovoid with karyosome. Capitulum present, small. Parabasal single, making four to twelve turns about axostyle. Pellicle thin.

Host.—*Glyptotermes parvulus* Sjöstedt, a Chilean termite.

Gigantomonas lighti sp. nov.

Diagnosis.—Shape pyriform.   Length 47 to 91μ, width 21 to 58μ.
Three anterior flagella fused in life to form a whip.   Posterior flagel-
lum borders external margin of large undulating membrane.   Anterior
and posterior medial edges of cresta reinforced by the rib-like thicken-
ings.   Nucleus pyriform, with karyosome, supported by capitulum.
Capitulum prolonged posteriorly as slightly chromophile core of axo-
style surrounded by fibrillar sheath.   Shield-like arrangement of
siderophile spherules adherent to left side of capitulum.   Parabasal
body single, cylindrical, with two marginal filaments, makes six to
twelve turns about fibrillar sheath.   Pellicle delicate.

Host.—*Kalotermes (Paraneotermes) simplicicornis* Banks, a ter-
mite from California and Arizona.

SUMMARY

1. The morphology and mitosis of *Gigantomonas lighti* sp. nov.,
a trichomonad flagellate from the intestine of *Kalotermes (Paraneo-
termes) simplicicornis* Banks, is described.   Particular attention is
paid to the dedifferentiation and redifferentiation of organelles at
mitosis.

2. In studying the fate and development of organelles it was found
that no one combination of fixative and stain gave entirely satisfactory
results for all organelles.

3. The importance of studying living material is stressed.

4. *Gigantomonas lighti* varies in length from 47 to 91μ and from
21 to 58μ in width.   The average size is $68 \times 38\mu$.   In shape it is roughly
pyriform.

5. Three anterior flagella are fused in life to form a whip while a
fourth, much longer and stouter than the rest, forms the intensely
staining external border of the undulating membrane.

6. The undulating membrane follows the external margin of the
intracytoplasmic, undulatory cresta to which its base is fused.

7. The flagella and cresta arise from a single chromatic granule,
the blepharoplast.

8. The blepharoplast and centrosome (kinetic and mitotic centers
of the cell) are closely associated but do not form a centroblepharo-
plast.

9. The pyriform nucleus, with karyosome, is enclosed by the capitulum of the axostyle which projects posteriorly beyond the nucleus as a core about which lies the fibrillar sheath of the axostyle.

10. The parabasal body, more highly developed than in any other known trichomonad, has a double origin, one marginal filament arising from the blepharoplast, and a second from the centriole. Its relation to the Golgi material of metazoan cells is refuted.

11. At the approach of mitosis, a fibrillar paradesmose forms between the divided centrioles which come to lie near its tips.

12. In the prophase the chromatin becomes organized into five double prophase chromosomes which tend to separate as the fibrils of the intranuclear spindle draw them toward the equatorial plate.

13. During the anaphase the five pairs of chromosomes, one pair of which is much smaller than the others, migrate toward the poles of the intranuclear spindle.

14. The body of the nucleus divides by simple constriction, the nuclear membrane persisting throughout mitosis.

15. All organelles of the neuromotor system are resorbed or discarded and new ones, including parabasal bodies and flagella, are redifferentiated.

16. The two new sets of organelles retain the common cytoplasmic mass until development is nearly complete and then the cell body quickly divides into two equal daughter cells.

17. *Myxomonas polymorpha* Dogiel is regarded as a synonym of *Gigantomonas*, as is also *Macrotrichomonas* Grassi. A comparative table, as well as diagnoses, of the recognized species of *Gigantomonas* is given.

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EXPLANATION OF PLATES

PLATE 8

All figures drawn with aid of camera lucida from permanent mounts × 1015.

Fig. 1 *Gigantomonas lighti* sp nov. Vegetative individual with an inter phase nucleus. After Schaudinn's fluid and Heidenhain's iron haematoxylin.

Fig. 2. Early prophase. Old parabasal body and cresta detached from neuromotor system. New parabasal bodies just beginning to grow out. After Zirkle's fluid and Heidenhain's iron haematoxylin.

Fig 3. Later prophase. Cresta resorbed Old parabasal body nearly resorbed. New parabasal bodies well developed. After Zirkle's and Heidenhain's iron haematoxylin.

Fig 4. Early anaphase. New marginal flagellum growing out from ble pharoplast. Old axostyle discarded by neuromotor system. From a Schaudinn's Heidenhain's iron haematoxylin preparation.

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PLATE 9

All figures drawn with aid of camera lucida from permanent mounts.

Fig. 5. A cytoplasmic parasite, perhaps a stage in the life cycle of *Sphaerita*, from a Zirkle's Heidenhain's iron haematoxylin preparation. × 1800.

Fig. 6. *Sphaerita* from a Zirkle's-Heidenhain's iron haematoxylin preparation. × 1800.

Fig. 7. A cresta torn from the neuromotor system in smearing. Fixed in Schaudinn's fluid and stained in Heidenhain's iron haematoxylin. × 1800.

Fig. 8. Capitulum drawn from left side showing the chromatic shield. A Schaudinn's-Heidenhain's iron haematoxylin preparation. × 1015.

Fig. 9. A portion of the parabasal body after Schaudinn's fluid and overstained in Heidenhain's iron haematoxylin.

Fig. 10. A portion of the parabasal body after Schaudinn's fluid and Delafield's haematoxylin.

Fig. 11. The fibrillar sheath of axostyle after osmic iodide, unstained. Capitulum does not brown, laminations of fibrillar sheath largely destroyed. × 1015.

Fig. 12. A double individual. Fixed with osmic vapor and stained with Heidenhain's iron haematoxylin. × 450.

Fig. 13. A reorganization individual just after plasmotomy, fixed in Zirkle's fluid and stained in Heidenhain's iron haematoxylin. × 1015.

Fig. 14. A telophase showing the new cresta. The capitulum is present, forming the core of the new axostyles. The old axostyle has not yet been cast out of the cytosome. After Zirkle's fluid and Heidenhain's iron haematoxylin. × 1015.

Fig. 15. A late anaphase. All old motor organelles, including flagella, have been discarded. Thus far only the parabasal bodies and flagella have been redifferentiated. From a Zirkle's fluid-Heidenhain's iron haematoxylin preparation. × 1015.
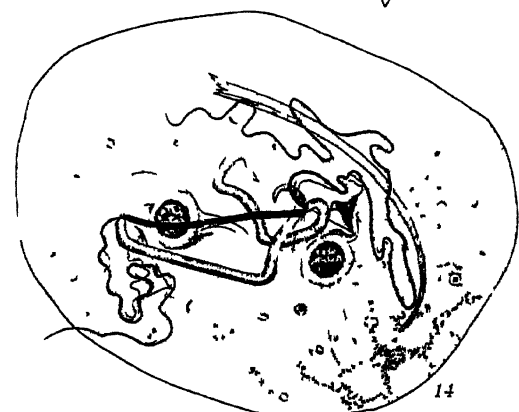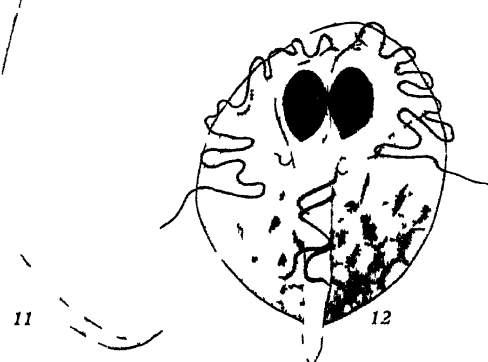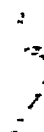
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FOUR NEW SPECIES
OF HAEMATOLOECHUS (TREMATODA)
FROM RANA AURORA DRAYTONI
FROM CALIFORNIA

BY

LLOYD G. INGLES

FOUR NEW SPECIES OF HAEMATOLOECHUS (TREMATODA) FROM RANA AURORA DRAYTONI FROM CALIFORNIA

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INTRODUCTION

The earliest mention of trematodes from the lungs of amphibia seems to be that made by Swammerdamm (1752) who described in his *Bibel der Natur* (translation of his "Biblia naturae, etc." 1737) a fluke which he saw in the lungs of a frog. This fluke, now known as *Haplotrema cylindracea* (Zeder), is one of the oldest known trematodes. Although trematodes from the lungs of *Anura* were described or mentioned in Europe in the early part of the nineteenth century by Zeder, Rudolphi, Dujardin, and others (Braun, 1889), it was not until the time of Looss (1894) that descriptions of specific value were given. Looss later (1899) gave the genus name *Haematoloechus* to this group. Now the name *Haematoloecha* had been given to an hemipteron by Stål. Looss, therefore, influenced by Braun, changed the name of the genus to *Pneumonoeces* (Looss, 1902) for this reason, namely: if family or subfamily names were ever formed from the generic names, the names of the two larger groups would be identical.

My attention has been called to the fact that this change by Looss is not in accord with the recommendation under Article 36 of the International Rules of Zoological Nomenclature. This recommendation states: "It is well to avoid the introduction of new generic names which differ from generic names already in use only in termination or in a slight variation in spelling which might lead to confusion. But when once introduced such names are not to be rejected on this account." After consulting the opinions of several systematists, it therefore seems to the writer best to restore the name *Haematoloechus* and reduce *Pnuemonoeces* to a synonym of that name.

In North America Leidy (1851, 1856) gave a few descriptions, which are now inadequate for specific determination, of frog lung

flukes. Stafford (1902) described five new species of lung flukes from Canadian frogs under the genus *Haematolocchus,* but later (1905) published the following names: *Pnuemonoeces longiplexus, P. breviplexus, P. varioplexus, P. similiplexus,* and *P. medioplexus.* Ward (1917) created the new genus *Pneumobites,* and included in it the first two of these species. As Stafford had only a few specimens of *Haematoloechus varioplexus,* and as it does not differ from *H. similiplexus* except in the size of the eggs, Cort (1915*b*) is inclined to consider *H. varioplexus* as a *species inquirenda.* Since eggs of a species might be variable (Cort 1915*a*), he is no doubt correct in considering it as such. Stafford (1905) also considered Pratt's *Ostiolum formosum* (1903) to be a synonym of *H. medioplexus.* Seeley (1906) described another lung fluke, *H. complexus* from *Rana pipiens* of North Carolina. Cort (1915*b*) described another species, *H. coloradensis,* from the same host-species from the east side of the Rocky Mountains in Colorado. The only other lung fluke that has been described in North America is Irwin's (1929) *H. parviplexus* from the *Rana clamitans* from Minnesota. To this list of five species of the genus *Haematoloechus* from North America the present paper adds four new species from the *Rana aurora draytoni* of California.

Three other species have been described, one by Klein (1905) who described *H. capyristes* from the *Rana hexadactyla* of India, another by Johnston (1912) who described *H. australis* from the *Hyla aurea* and the *Limnodynastes peronii* of Australia, and the third, by Travassos and Artigas (1927) under the name *Pneumonesces* (ac. *Pneumonoeces*) *neivai,* from *Leptodactylus oscellatus* of Brazil.

The writer began work on the trematodes of the Californian frogs and reptiles during the summer of 1929 while he was a graduate student at the University of California. At present, work on the life-histories of three forms is in progress, and it will be published later.

The writer wishes to express his appreciation to Professor C. A. Kofoid for his helpful advice and for use of his library during the course of this work. Thanks are also due Drs. S. S. Berry, A. S. Campbell, Jean M. Linsdale, Tracy Storer, S. J. Holmes, Joseph Grinnell, and Mr. Arthur Cohen who have helped with advice or material.

The type in each case is deposited in the U. S. National Museum and the cotypes in the collection at the University of California at Berkeley.

DESCRIPTION OF SPECIES

Haematoloechus kernensis sp. nov.

Diagnosis.—Rather long, slender worms; widest at the level of the testes; no spines; ratio of the oral sucker to the acetabulum about 1:1; ovary and testes not lobed; acini of the vitellaria large with 6 to 20 in a group; longitudinal folds of the uterus outside of the intestinal caeca not extending beyond the level of the posterior testis; eggs average 30μ in length by 16μ in width: from the lungs of *Rana aurora draytoni* from Bakersfield, Kern County, California.

Description.—This species of fluke was found in the lungs of *Rana aurora draytoni* which was taken from a small stream near Bakersfield, California (fig. 1). It was the only frog of the many that were examined from this station, that was infected with this species of fluke, and it harbored ten mature worms. *H. kernensis* was later taken from the same species of frog from an irrigation ditch near Bakersfield, California, which is only a few miles from the original station.

The worms of *H. kernensis* are rather slender and are over four times as long as wide. They taper gradually from the level of the posterior testis to the oral sucker which is slightly subterminal. Behind the posterior testis the body tapers more rapidly to a rounded end. The following measurements were all made from mounted material. Average length of thirteen worms 6.3 mm.; average width of body at the widest point 1.54 mm. The shortest worm was 5.5 mm., the longest 7. mm.

The subterminal oral sucker is nearly circular and has a diameter in nine specimens of 0.44 mm. It communicates directly with a strong pharynx which has an average diameter across its length of 0.31 mm. A collar of deeply stained tissue between the pharynx and the oral sucker could be seen in all the mounts, and is no doubt a part of the nervous system. A short oesophagus connects the pharynx to the rather large intestinal caeca which extend nearly to the posterior end of the body. The acetabulum averages a little less in diameter than the oral sucker. The average diameter of the acetabulum in ten individuals was 0.44 mm. It is nearly circular and is located in the posterior end of the first half of the body. The ratio of the oral sucker to the acetabulum averages practically 1:1 in ten individuals: in all the mounts it varied from 7:6 to 15:16. The average ratio of the oral sucker to the pharynx is 3:2.

The cuticula of *Haematoloechus kernensis* is entirely smooth, there being no indication of spines at any place.

The ovary is an elliptical or oval organ which lies at one side of the body just behind the acetabulum. Its longest diameter averaged 0.58 mm. in six individuals, and the width averaged 0.37 mm. In some individuals it was almost spherical. The seminal receptacle is a somewhat larger organ lying partly ventral and posterior to it. The connections of the ovary, seminal receptacle, and the uterus were similar to those described for *Haematoloechus oxyorchis* (below), but they

differ in having a larger common vitelline duct or yolk reservoir which is ventral to the seminal receptacle, and which enters the uterus in a different manner. The first part of the uterus has a very thick hyaline wall for some distance (fig. 7). At first the uterus runs forward a short distance looping over the anterior edge of the ovary and the back part of the acetabulum. It then winds ventrally and posteriorly between the testes to the end of the body. In this region it winds about with no definite pattern until it fills all the space as far anterior
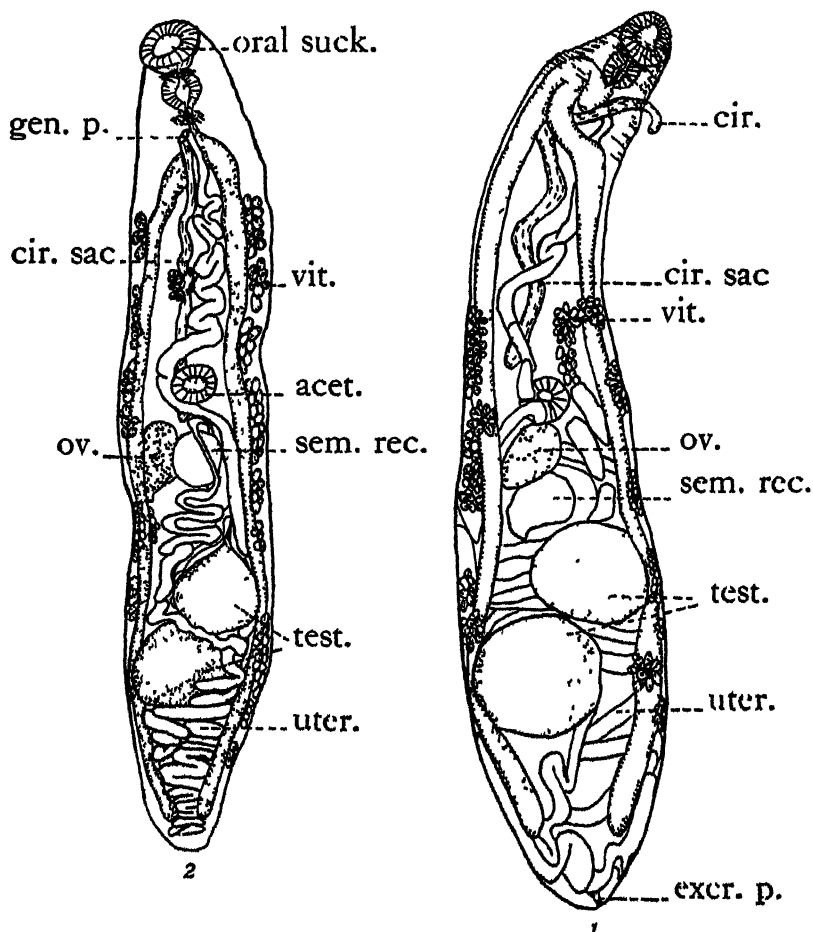
Fig. 1. *Haematoloechus kernensis* sp. nov. Dorsal view; eggs in uterus omitted for the sake of clearness. Drawn with aid of camera lucida; × 13. *cir.,* cirrus; *cir. sac,* cirrus sac; *vit.,* vitellaria; *ov.,* ovary; *sem. rec.,* seminal receptacle; *test.,* testes; *uter.,* uterus; *excr. p.,* excretory pore.
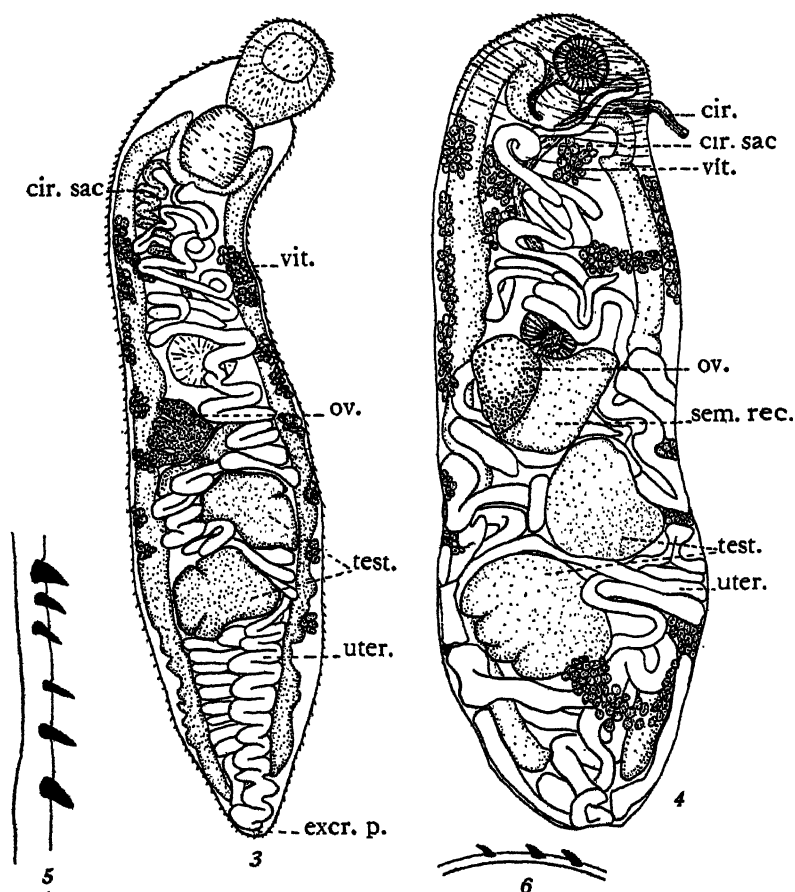
Fig. 2. *Haematoloechus oxyorchis* sp. nov. Ventral view; eggs in uterus omitted for the sake of clearness. Drawn with aid of camera lucida; × 15. *oral suck.,* oral sucker; *vit.,* vitellaria; *acet.,* acetabulum; *sem. rec.,* seminal receptacle; *test.,* testes; *uter.,* uterus; *ov.,* ovary; *cir. sac.,* cirrus sac; *gen. p.,* genital pore.

as the posterior testis. It then starts in an anterior direcion, dorsally to the folds of the descending uterus. It goes between the testes again and winds about to the genital pore which is in the midline of the body ventral and slightly posterior to the edge of the pharynx. The eggs of this species were brown, and had an average length of 30μ with an average width of 16μ. They varied in length between 25μ and 37μ.

The testes are large, almost spherical organs that lie on either side of the body some distance behind the ovary. They are nearly the same size in all the specimens. The average diameter of the anterior one in eight specimens was 0.97 mm., while that of the posterior one was 0.96 mm. The ducts from them could not be traced forward to the cirrus sac, which was long, and, when well extended, reached to or slightly beyond the acetabulum. In most of the well extended specimens the cirrus was everted (fig. 1). The vitellaria are mostly extra-caecal in position. Anterior to the acetabulum there may be from one to three groups of acini that are inter-cecal. Behind the testes there are no groups between the caeca, but opposite the posterior testis is a group dorsal to the caecum which may extend over into this area. The acini occur in definite groups ranging from 6 to 20 to the group.

Comparisons—*Haematoloechus kernensis* is most closely related to *Haematoloechus parviplexus* Irwin. It has the same shape of body and general arrangement of uterus as that species. The range of egg size and the ratio of the oral sucker to the pharynx agree in both species. *H. kernensis* differs from *H. parviplerus*, however, in lacking spines, in the ratio of the oral sucker to the acetabulum, and in the shape of the ovary and the testes. *H. kernensis* differs from *H. tumidus* (below) in smaller size, in shape of body, in having no spines, in ratio of oral sucker to pharynx, and in shape of ovary and testes. It agrees with later species in type of uterus, range of egg size, and in having an acetabulum nearly as large or larger than the oral sucker.

Haematoloechus oxyorchis sp. nov.

Diagnosis. Medium sized species; cuticula free from spines; ratio of the oral sucker to the acetabulum 5:4; ovary somewhat lobed; testes unlobed and coming to an acute point on the anterior side; cirrus sac long, extending beyond to the region of the acetabulum; vitellaria of rather large acini with from 9 to 14 in a group; no folds of the uterus outside of the intestinal caeca; eggs average 27μ in length and 17μ in width; from the lungs of *Rana aurora draytonii* from Oakland, California.

Description.—This is the most common species of lung flukes to be found in *Rana aurora draytonii* from the San Francisco Bay region of California. Nearly one-half of the adult frogs that were examined from this region were infected. It was not found in any of the frogs that were examined from Bakersfield, California.

The worms taper gently to both ends and average more than five times as long as wide (fig. 2). The cuticula is free from spines. The average length of twenty worms in my collection is 5.8 mm., and the average width is 0.87 mm. The length of the shortest mature worm is 3.0 mm., and that of the longest is 6.5 mm.

The campanulate oral sucker is terminal or slightly subterminal in position. It averages 0.41 mm. in diameter if the worms have not been flattened out by pressure. The strong muscular pharynx is nearly spherical with the diameter averaging 0.31 mm. in nine individuals. It is bounded anteriorly and posteriorly by rings of cells which might be parts of the nervous system or glands, or both. The pharynx is followed by an oesophagus which is about equal to its own length. The oesophagus leads into two large caeca which extend nearly to the posterior end of the body. The circular acetabulum is nearly in the middle of the body. It averaged 0.32 mm. in diameter in ten individuals. The ratio of the oral sucker to the pharynx is 4:3 while the average ratio of the oral sucker to the acetabulum is 5:4. This latter ratio varies in individuals, however, from 7:6 to 8:5.

The ovary in this species is usually slightly lobed (fig. 9). It is located behind the acetabulum at the anterior end of the posterior half of the body. It is somewhat variable in outline and averaged 0.58 mm. in length and 0.31 mm. in width in ten specimens. Its relation to the seminal receptacle, vitelline ducts, Mehlis' gland, and the uterus is also shown (fig. 9). Lying beside or behind the ovary and ventrally to it is the seminal receptacle. It is usually oval in shape and about the size of the ovary. The uterus leaves the female organs, and after running slightly anterior, starts looping its way posteriorly between the two testes to the posterior end of the body, where it winds its way back, coming between the testes again and continues its way to the genital pore. The genital pore is slightly to one side of the midline of the body and anterior to the place where the caeca join the oesophagus (fig. 2). The "figure 8" shows the relation of the metraterm to the cirrus sac. The eggs are brownish in color, and have an average length of 27μ and an average width of 17μ. The length varies from 26μ to 30μ. The testes lie some distance behind the female organs on either side of the body. They are drawn out to an acute point anteriorly where they join their vas efferens, which gives each of them the shape of an inverted turnip. If not distorted by pressure, this shape is constant in all the individuals that have been examined. The size of the testes is quite variable. The anterior testis averaged 0.76 mm. in diameter while the posterior one averaged 0.78 mm. Ten worms were used in these measurements. The tubes from the testes to the cirrus sac could not be followed in any of the mounts. The cirrus sac, as mentioned before, is long and opens through the genital pore which is slightly to one side of the midline of the body. Although the position of the genital pore in *Haematoloechus complexus* was given as lateral to the midline by Seeley (1906), it was questioned by Cort, who ascribed this position on Seeley's drawing to a mistake in observation (Cort, 1915*b*). This character seems to be constant and noticeable, however, in this species when straight and flattened mounts are available.

The vitellaria are almost entirely extra-caecal in location. In some specimens, however, from one to three groups may be inter-caecal and anterior to the acetabulum. Such a group is shown, although the figure is a ventral view, and the acini of this group are dorsal in position (fig. 2). Dorsally and posteriorly to the left testis the acini extend slightly over into the inter-caecal region. A peculiarity about the

species is the presence of the acini on the ventral surface of the two caeca. In all the mounts this is a constant feature. The acini are somewhat larger anteriorly and are arranged in groups from 9 to 14. The vitelline collecting ducts are large and clearly discernible (not shown in fig. 2).

Comparisons.- -Haematoloechus oxyorchis seems to be more nearly related to *H. complexus* and *H. confusus* (below) than any of the other described species. It differs from the former in having smaller eggs and an acute anterior projection on each of the testes. It differs from the latter in being spineless, in the shape of the testes, and in its larger average size. It agrees with *H. complexus* in the tendency of the ovary to be lobed, in not being spiny, in type of uterus, in size and shape of body, and in the ratio of the oral sucker to the acetabulum. It agrees with *H. confusus* in the range of egg size, in the ratio of the oral sucker to the acetabulum, and in type of the uterus.

Haematoloechus confusus sp. nov.

*Diagnosis.--*Length over four times as long as wide; spines present all over the body; ratio of oral sucker to acetabulum 4:3; field of the genital glands without vitellaria about one-third body length; ovary and testes irregular in form and lobed; few vitellaria between the intestinal caeca; no longitudinal folds of the uterus outside of the intestinal caeca; eggs average 26μ in length and 15μ in width; habitat, lungs of *Rana aurora draytonii* from Oakland, California.

Description.- Haematoloechus confusus was found in the lungs of a single specimen of *Rana aurora draytonii* from the Thornhill Pond in Oakland, California. Thirteen mature flukes were present in this individual. This species of fluke is apparently not very common in this locality since it has not been recorded in numerous examinations that were made on this species of frog in this and other parts of California.

In life the worms were long and spindle shaped, tapering immediately and sharply behind the ends of the intestinal caeca (fig. 3). The largest mount measured 4.9 mm. in length while the length of the smallest worm was 3.3 mm. The average for eight well extended specimens was 3.9 mm. in length and 0.9 mm. in width. The entire body is covered with spines, which are much larger and coarser at the anterior end (fig. 5). The largest of these spines had a length of 14μ but they were much smaller and fewer at the posterior end of the body. The oral sucker is terminal and pear-shaped with an average length of 0.44 mm. and an average width of 0.46 mm. It is followed by a strong pharynx which has an average length of 0.34 mm. and an average width of 0.28 mm. The ratio of length of the oral sucker to length of the pharynx is about 4:3. The pharynx is followed by a short oesophagus (not shown in figure) which soon branches to form the rather large intestinal caeca which extend nearly to the posterior end of the body. These caeca were greatly distended with the frog's blood when the worms were first removed from the lungs. After fixing and staining, the caeca shriveled and presented an irregular outline in the mounted material.

Fig. 3.   *Haematoloechus confusus* sp. nov. Dorsal view; eggs in uterus omitted for the sake of clearness.   Drawn with aid of camera lucida; × 28.   *cir. sac,* cirrus sac; *vit.,* vitellaria; *ov.,* ovary; *test.,* testes; *uter.,* uterus; *excr. p.,* excretory pore.

Fig. 4.   *Haematoloechus tumidus* sp. nov.   Dorsal view.   Drawn with aid of camera lucida; × 12.   *cir.,* cirrus; *cir. sac,* cirrus sac; *vit.,* vitellaria; *ov.,* ovary; *sem. rec.,* seminal receptacle; *test.,* testes; *uter.,* uterus.

Fig. 5.   Spines near anterior end of *H. confusus.*   Drawn with aid of camera lucida; × 466.

Fig. 6.   Spines near anterior end of *H. tumidus.*   Drawn with aid of camera lucida; × 466.
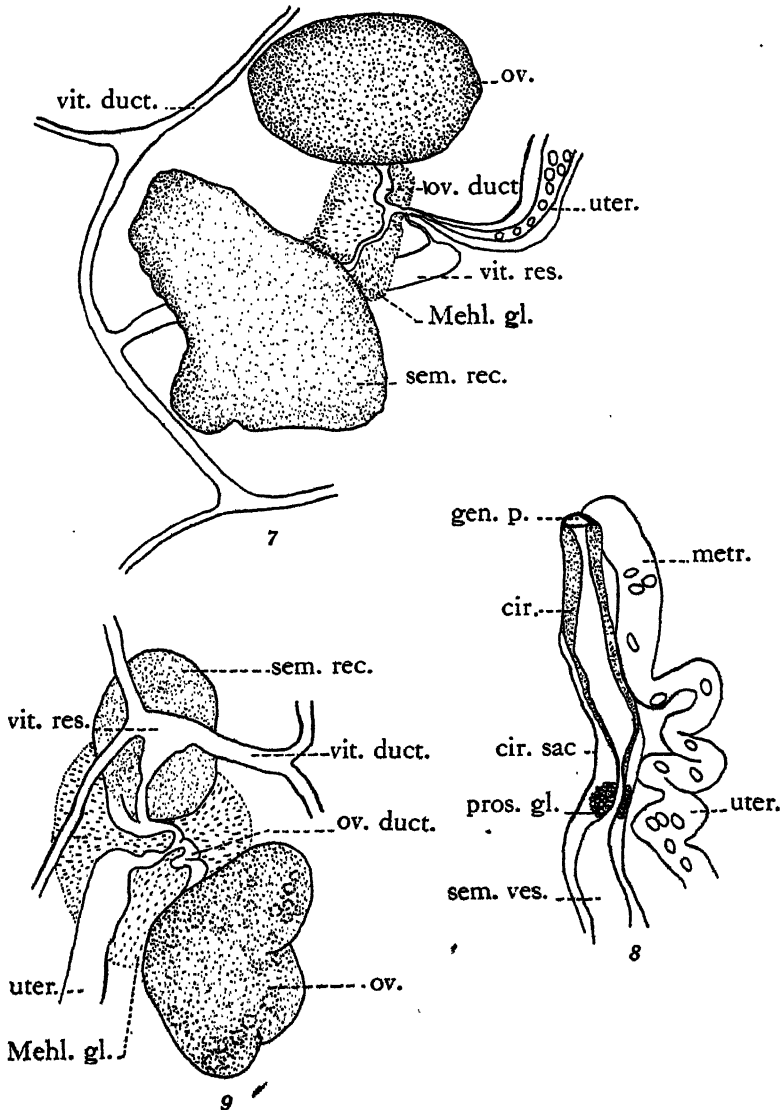
Fig. 7. Female reproductive organs of *Haematoloechus kernensis*. Dorsal view. Drawn with aid of camera lucida; × 70. *vit. duct.*, vitelline duct; *ov.*, ovary; *ov. duct.*, oviduct; *uter.*, uterus; *vit. res.*, vitelline reservoir; *Mehl. gl.*, Mehlis' gland; *sem. rec.*, seminal receptacle.

Fig. 8. The metraterm and cirrus of *Haematoloechus oxyorchis*. Drawn with aid of camera lucida; × 70. *gen. p.*, genital pore; *metr.*, metraterm; *cir.*, cirrus; *cir. sac*, cirrus sac; *pros. gl.*, prostrate gland; *uter.*, uterus; *sem. ves.*, seminal vesicle.

Fig. 9. Female reproductive organs of *Haematoloechus oxyorchis*. Drawn with aid of camera lucida; × 70. Dorsal view; *sem. rec.*, seminal receptacle; *vit. duct*, vitelline duct; *ov. duct*, oviduct; *ov.*, ovary; *Mehl. gl.*, Mehlis' gland; *uter.*, uterus; *vit. res.*, vitelline reservoir.

The acetabulum is nearly circular and has an average diameter of 0.32 mm. The average ratio of the oral sucker to the acetabulum is about 4:3, but varies in individuals from 5:4 to 9:5.

The ovary of this species is distinctly and irregularly lobed. In some mounts it is three times longer than wide, but in others (fig. 3) it is nearly as wide as long. In eight mounts it averaged 0.40 mm. in length by 0.19 mm. in width. The distance from anterior edge of the ovary to posterior edge of the hind testis is slightly over one-third of the body length, but in some mounts the genital organs occupy much less space. Behind and somewhat lateral to the ovary is the seminal receptacle, which is irregular in shape and usually about the size of that organ. The testes, like the ovary, are both lobed and are irregular in outline. They are nearly the same size. The front one had an average length, in the eight specimens that were measured, of 0.51 mm. and an average width of 0.44 mm. while the hind one averaged 0.50 mm. in length and 0.42 mm. in width. They are usually contiguous or nearly so. So far as the ducts of the reproductive organs could be made out, they are similar to those that have been described for other species of this genus. The cirrus sac is much folded and coiled anterior to the acetabulum, and, even then, usually extends posterior to it.

The vitellaria are almost entirely to be found in the lateral zones outside of the intestinal caeca. There are individuals in which one or two groups of acini are nearly entirely inter-caecal however. The acini are arranged in groups of 4 to 20.

The uterus leaves the region of the ovary and seminal receptacle, and goes to the opposite side of the body where it folds back upon itself, then turns, posteriad and ventrad, to go between the testes. It then loops from side to side until it reaches the posterior end of the body where it rises to the dorsal side, and winds its way back between the testes; hence, with many irregular convolutions and loops, it goes forward to the genital pore, which is nearly in the midline of the body beneath the fork of the intestinal caeca. There are no longitudinal folds of the uterus outside of the intestinal caeca (fig. 3). Measurement of a large number of eggs from eight individuals gives a variation in length from 24μ to 29μ. The average length is 26μ and the average width is 15μ. They are dark brown in color.

Relationships.—*Haematoloechus confusus* more closely resembles *H. complexus* than any other described lung fluke. It agrees favorably with this latter species in size, type of uterus, the lobed testes and ovary, and in ratio of the oral sucker to the acetabulum. It differs from H. complexus, however, in having spines, in having smaller eggs, in the arrangement of the vitellaria, and in the testes and ovary always being lobed. It agrees with *H. coloradensis* in arrangement of the vitellaria, in type of the uterus, and in the presence of spines, although the latter character differs somewhat in the distribution of the two worms. It differs from this latter species in having shorter eggs, in the shape of ovary and testes, and in the different ratio of the oral sucker to the acetabulum.

Haematoloechus tumidus sp. nov.

Diagnosis.—Has usual characters of the genus except the acetabulum is larger than the oral sucker; large thick spines and only on the anterior end; testes oval or lobed; vitellaria of variable sized acini with six to thirty-one in a group; longitudinal folds of the uterus extending outside of the caeca up to posterior limit of posterior testis; eggs average 32μ in length and 17μ in width; from the lungs of *Rana aurora draytonii.*

Description.—Eight specimens of this large species were taken from the lungs of the California red-legged frog, *Rana aurora draytonii,* during the first part of March 1931. The frog was brought to the laboratory by Mr. Arthur Cohen, who had collected it from an irrigation ditch in the city of Bakersfield, California. A week later another frog was obtained from the same general locality, and was found to harbor two specimens of the same fluke. Many other records are now at hand which indicate that this species is very common in this locality.

The worms are long, and blunt at both ends (fig. 4). When removed and placed in a 0.7% saline solution, they swayed the anterior end about very slowly. The animal was held to the substratum by the acetabulum, and occasionally it would try to attach itself by the oral sucker. During this attempt to attach the oral sucker, the pharynx would pulsate strongly. Measurements were made of the length and width of the living animals, the longest of which when greatly extended, measured 18 mm. and 0.51 mm. wide. After mounting it was found that all the specimens were more or less contracted, but even then, the body length was found to average 8.1 mm. and the width to average 2.5 mm. The shortest worm was 6.4 mm., and the longest one was 9.7 mm., and even these were somewhat contracted. From these measurements it is easy to see that this species is of very large size, and is probably the largest fluke described from North America belonging to this genus.

The oral sucker is round and subterminal, and in one still living, medium-sized individual, had a diameter of 0.41 mm., but when the worms were flattened out beneath a coverglass and were fixed, their oral suckers averaged 0.61 mm. in diameter. The pharynx in a living medium-sized individual measured 0.47 mm. in length by 0.39 mm. in width. After flattening and staining they averaged 0.49 mm. in length. Because of the contracted state of the material the length of the oesophagus could not be determined accurately, but it appeared to be about the length of the pharynx. The large caeca extend nearly to the posterior end of the animal. The acetabulum is larger than the oral sucker both in life and after fixing the specimens. In a living specimen it measured 0.47 mm., and after flattening and mounting it averaged 0.68 mm. in diameter. The ratio of oral sucker to acetabulum was 5 : 6 in both living and preserved specimens. This species differs in this respect from all the described species of the genus. The ratio of oral sucker to pharynx was 1 : 1 in life, but after mounting it was 5 : 4 because of the greater spreading of the oral sucker in flattening the worms. Spines could be seen at the anterior end while the animal

was living, but after mounting only a few scattered ones could be recognized. The arrangement and shape of the spines approximate those of *H. coloradensis* (fig. 6). The excretory vesicle could not be recognized in any of the specimens.

The ovary in this species varies from slightly oval to kidney-shaped, but is never lobed. Its average length in six flukes was 0.87 mm. Its long axis is more or less parallel with the axis of the body. The seminal receptacle is a much larger organ lying laterally and somewhat posteriorly to the ovary. It is more or less irregular in shape. The vitellaria consist of irregularly shaped groups of acini of quite variable size. They are distributed dorsally and laterally to the caeca from the lower level of the posterior testis to nearly the level of the genital pore. Behind the posterior testis are thirty to forty acini between the caeca. In front of the acetabulum also, are three to four groups of acini between the caeca. The acini vary in the groups from six to thirty-one. The testes are somewhat irregular ovals in shape, and may or may not be lobed. Their longitudinal axis is seldom parallel with that of the body. They are usually abut, but never on opposite sides at the same level. The average of the greatest diameters of the anterior testes was 0.16 mm. while that of the posterior ones averaged a trifle smaller or about 0.15 mm. Their shape and size are quite variable depending on the size of the individual. In some specimens could be seen the vasa efferentia which left their anterior part and joined the lower part of the cirrus sac at about the level of the acetabulum. Whether or not they joined before entering the cirrus sac could not be determined with certainty. The cirrus was quite often protruded when the worms were placed in the fixative (fig. 4). The eggs averaged 32μ in length by 18μ as they were laid. After mounting, twenty eggs, in the metraterms of all specimens, averaged 32μ by 17μ in width with a variation in length between 30μ and 36μ. The eggs were brownish in color, and the developing miracidium presented a characteristic figure. The course of the uterus in this species was difficult to trace because of the great number of folds, and also because most of the eggs were shed from it before the specimens were fixed. By studying all of the material, however, its course was made out from ovary to genital pore. After leaving the female organs, the uterus runs forward a short distance, then looping over itself comes back ventrad to the ovary and seminal receptacle, hence it winds from side to side posteriorly between the testes, behind which the coils are irregular and torturous. In this region the coils run anteriorly outside of the caeca as far as the testes, then they return to the posterior end, hence forward between the testes, then laterally to the side opposite the ovary, from which position it goes forward making about four coils until it reaches the level of the acetabulum. Between the acetabulum and the narrow metraterm, it makes about six loops from side to side between the caeca.

Relation.—*Haematoloechus tumidus* differs from all the previously described species of this genus in being larger, and in having the acetabulum larger than the oral sucker. It differs from *H. similiplexus* in that the longitudinal folds of the uterus outside of the caeca are not so extensive, and in having smaller eggs. It differs from *Pneumono-bites breviplexus* in shape of the testes, in having spines, and in shape

of the ovary. It is like this latter species in size and the superficial arrangement of the vitellaria. It differs from *Pneumonobites longiplexus* in the shorter extent of the uterus outside of the caeca, in size and shape of the ovary and testes, and in size of the eggs. It is like *breviplexus* in having spines, and in the general distribution of the vitellaria. It differs from *Haematoloechus parviplexus* in shape of the testes, in having larger eggs, in shape of the ovary, and above all in the different ratio of the oral sucker to the acetabulum. It agrees with this species in the type of uterus, presence of spines, and general size. It differs from *Haematoloechus kernensis* in its larger size, in having spines, and in the difference in shape of the ovary and testes. It is similar to *kernensis* in type of the uterus, and in having the acetabulum larger or nearly equal to the oral sucker.

SUMMARY

This paper describes and gives figures of four new species of frog lung flukes of the genus *Haematoloechus*, which were collected from the California red-legged frog, *Rana aurora draytonii*. These flukes have been compared with previously described species, and have been shown to be specifically distinct.

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CEPHALOGONIMUS BREVICIRRUS, A NEW SPECIES OF TREMATODE FROM THE INTESTINE OF RANA AURORA FROM CALIFORNIA

BY

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HISTORY

The genus *Cephalogonimus* was established by Poirier (1885) with *C. lenoiri* as the type species. The flukes were collected from the turtle, *Tethrathyra vaillantii* from Senegal. He defines the genus as, "Distomes à orifices génitaux situés à la partie antérieure du corps, sur la face dorsale, un peu en avant de la ventouse oral." Looss (1899) in speaking of this point in his definition of the genus says: "Genitalporus am Vorderende, auf der Spitze einer kleiner, über dem Mundsaugnapf sich erhebenden Kuppe." Thus with respect to the exact position of the genital pore there is a slight disagreement, but since there are now known species of this genus which fit one or the other of these definitions it seems that both should be included in the present conception of the genus.

Nine species have been assigned to this genus. Of these *C. trachysauri* MacCullum should, according to Chandler (1923), undoubtedly be placed in a new genus because of its many divergent anatomical features. Stafford (1902) considered Leidy's *Distomum retusum*, which Leidy had referred to Dujardin's type, as being synonomous with *C. americanus* Stafford. Chandler (1923) believes that *C. retusus* is identical with *C. europaeus* Blaizot. Excluding *C. trachysauri* and *C. retusus* there remain in the genus seven described species and to this list the present paper adds the eighth, *Cephalogonimus brevicirrus*, from the intestine of *Rana aurora draytoni* from California. Four American species have been described. Stafford (1902) described *C. americanus* from *Rana virescens* and possibly also from *Rana clamata* from Canada. Nickerson (1912) described *C. vesicaudus* from the intestine of two soft-shelled turtles, *Aspidonectes* and *Amyda*

from Minnesota. Chandler (1923) described *C. amphiume* from the intestine of *Amphiuma means.* Stunkard (1924) described *C. compactus* from the Floridan turtle, *Pseudemys floridana.* In addition to these American species two others have been described besides the type, *C. leniori.* These are *C. europaeus* Blaizot (1910) from the European frog, *Rana esculenta,* and *C. emydalis* Moghe (1930) from the tortoise, *Emydra granosa* from India.

DESCRIPTION

The first individuals of this fluke to come to my attention were from the intestine of a large specimen of *Rana aurora draytoni* from near Bakersfield, California. This specimen was collected from an irrigation ditch on March 10, 1931. It harbored about fifty individuals of *Cephalogonimus brevicirrus* as well as six individuals of a species of *Gogoderina,* one *Haematoloechus,* and several individuals of an unidentified nematode. After the living flukes had been measured and studied they were fixed in hot Bouin's fluid and some stained with Mayer's hemalum, others with alum cochineal. The ones that were sectioned were stained in the bulk with Delafields haemotoyxlin.

The shape of the worms was somewhat variable but usually they were about one-third as wide as long and were more pointed anteriorly than posteriorly. The posterior end was often quite truncate especially in the living condition. In many mounted specimens the posterior end was actually indented at the opening of the excretory pore.

The average size of five mounted specimens was 2.09 mm. in length and 0.64 mm. in width. The size of the type (fig. 1) was 2.25 mm. in length and 0.71 mm. in width. One worm which had a width in cross-section of 0.57 mm. measured 0.34 mm. in thickness. The thickness varies greatly, however, and some of the flukes were nearly circular in cross-section. The oral sucker is larger than the acetabulum. In the mounted specimens the former averaged 0.21 mm. across its longest diameter and the latter averaged 0.17 mm. The fixing seems to have caused considerable shrinking, for in the living material these organs measured 0.26 mm. and 0.21 mm. respectively for one medium sized individual. The oral sucker is subterminal and the ventral sucker is well forward in the first half of the body.

The cuticle at the level of the first testis is 6.5μ thick. It is provided with spines which disappear entirely behind the level of the posterior testis. These spines are larger anteriorly and diminish in

size posteriorly. At the posterior end of the oesophagus on the dorsal side they measure 11.6μ in length. They were larger on the dorsal side than elsewhere.

Behind the oral sucker is a prepharynx and this is followed by a pharynx which is wider than long. The average width of the pharynx is 0.08 mm. and the length is 0.05 mm. in mounted specimens. The pharynx is followed by an oesophagus which averaged 0.12 mm. in length. The intestinal caeca extend as a rule but little behind the level of the posterior testis. In some individuals they do not quite reach the margin of the posterior testis. The caeca vary in thickness in the different individuals depending on the amount of material in them. In cross-sections the nervous system could be seen as a horseshoe-shaped body over the posterior part of the oral sucker.

The posterior part of the excretory system could be seen in some of the whole mounts but not with distinctness. It was therefore necessary to follow this system by means of serial sections. The excretory pore is located medially at the bottom of a dent at the posterior end of the body (pl. 10, fig. 1). This pore opens anteriorly into a peculiar oval-shaped body, the "caudal vesicle" of Nickerson (figs. 1 and 5). The inside of this vesicle is made up of infoldings of the epithelium, which appear like clear spines when seen in the whole mounts. This peculiar structure has been described for but one other trematode, which is also in this genus, *C. vesicaudus* Nickerson (1912). The caudal vesicle of *C. brevicirrus* is not so well developed nor so large as it is in *C. vesicaudus*. Anterior to the caudal vesicle and dorsal to the folds of the uterus the excretory system consists of a large central stem which bifurcates behind the posterior testis. This stem gives off two main branches which extend anteriorly up to the level of the acetabulum in the two flukes that were sectioned serially. The main stem also gives off lateral branches which in turn subdivide. The reconstructed diagrams of the excretory system based on serial sections of these flukes show that it is not symmetrical and is unlike in the two individuals. A few of the whole mounts also show this peculiarity but not clearly. This condition (figs. 2 and 3) seems to be quite different from that described for the other species of the genus. Nickerson (1912) figures a reconstructed excretory system of *C. vesicaudus*. In that species the two main branches extend to the anterior end of the body, and since he has drawn the lateral branches from the main stem for only one side of the body, it is assumed that he found them symmetrically arranged.

The large branches of the excretory system are lined with very large, irregular, columnar, epithelial cells. Throughout the entire length of the excretory system these cells are in evidence and they project into the lumen independently of one another, which Nickerson (1912) thinks might suggest amoeboid activity during life (fig. 4). The caudal vesicle is surrounded outside by large oval cells with large, deeply-staining nuclei, while the cytoplasm is clear and non-granular (fig. 5). Such cells are not to be found surrounding other parts of the excretory system.

The ovary is slightly oval and is situated on the right or left side of the acetabulum. Its anterior edge is about on the level with the anterior margin of the acetabulum, or is anterior to it. In fifteen individuals it was located on the left side eight times and on the right side seven times. As measured in five mounted specimens, it averaged 0.18 mm. in length by 0.15 mm. in width but in one medium-sized specimen in life it measured 0.24 mm. by 0.20 mm. after being flattened out somewhat by the coverglass. Shortly after the oviduct leaves the ovary it is joined by a duct from the seminal receptacle, a spherical body lying posterior and often dorsal to it. A little farther along it receives the common duct from the vitellaria. Laurer's canal can be traced easily in the sections. It rises from near the place where the duct from the seminal receptacle joins the oviduct and runs posteriorly to the dorsal wall (fig. 4). The uterus could be traced with difficulty since in most of the whole mounts the whole posterior part of the body was filled with eggs, and its walls were very difficult to see, but the figure 1 represents the condition as it exists in the type. The uterus passes between the two testes and winds posteriorly on the left side and anteriorly on the right side to the genital pore, which is just above the center of the oral sucker.

The seminal receptacle varies in size but is usually circular in shape and the sperm in it are arranged in such a manner as to give it the appearance of a vortex (figs. 1 and 4). The Mehlis glands are surrounding the ootype on the side opposite the ovary but these structures are to be seen only with difficulty in the whole mounts and in figure 4 they are not seen in this relation because the section was not cut at the proper level to show this feature.

The anterior testis is situated close behind the acetabulum on the side opposite the ovary with its outer wall extending over the caecum on that side. It is smaller than the posterior testis and averages 0.25 mm. in diameter in the mounted specimens. The posterior testis is on

the same side as the ovary and averages 0.28 mm. in diameter. The vas efferens is to be seen running from the anterior edge of each testis to the posterior edge of the cirrus sac. There is a small vesicle formed in each of the ducts at about the level of the acetabulum (fig. 1).

The cirrus sac is perhaps the most distinctive feature of this species. It is comparatively short and extends as a rule only a little beyond the forking of the intestinal caeca. It was with reference to this distinct morphological feature that the name *brevicirrus* was chosen. The seminal vesicle is two-lobed and is situated in the posterior end of the cirrus sac. The part of the sac anterior to the seminal vesicle is thickened and contains the prostrate glands.

The vitellaria are both inter-caecal and extra-caecal in position. On the side next to the ovary they extend farther anteriorly than on the opposite side but not so far posteriorly (fig. 1). The common vitelline ducts enter the oviduct just a little distad from the place where the Laurer's canal joins it.

RELATIONSHIPS

Cephalogonimus brevicirrus is perhaps more closely related to *C. americanus* than any other described species. It is like this species in that it has a frog host; the size and shape agree in both; the oral sucker is larger than the acetabulum; the testes are not in the midline of the body; the distribution of the spines is similar; both have a relatively long oesophagus; both are alike in the position of the genital pore; and both species show amphitypy with respect to the ovary. The size of the eggs is nearly the same in both species. It is unlike *C. americanus* in having a shorter cirrus sac; in having a caudal vesicle; in the distribution of the vitellaria; in the farther anteriorly situated ovary with respect to the ventral sucker; in having in each of the vasa efferentia a small vesicle; and the testes are more nearly the size of the ovary in *C. americanus* than they are in *C. brevicirrus*.

In having a caudal vesicle the fluke resembles *C. vesicaudus*, but differs from it in having the oral sucker larger than the ventral sucker; in having an oesophagus; in having a different position of the genital pore; and the shapes of the ovaries and the testes are different in the two flukes.

Another fluke which closely resembles this species is *C. europaeus* Blizot (1910). The position of the ovary with respect to the ventral sucker; the relatively long oesophagus; the relative size of the suckers; the distribution of the spines, and other features agree in the two species. *C. brevicirris* differs from *C. europaeus* in having a shorter *cirrus* sac; in having a pharynx; in having the vitellaria more anteriorly situated; and in the different arrangement of the excretory system and the uterus.

The type is deposited in the United States National Museum and the paratypes are in the collection of the University of California.

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EXPLANATION OF PLATE 10

Fig 1   *Cephalogonimus* brevicirrus n sp   Dorsal view, × 41

Figs 2 and 3   Freehand reconstruction of the excretory system from sections of two individuals of *C brevicirrus*

Fig 4   Cross section of *C brevicirrus* at the level of the anterior testis and the ovary   × 120

Fig 5   Cross section through the caudal vesicle of *C brevicirrus*   × 313

gen p, genital pore, *sem ves*, seminal vesicle, *ov*, ovary, *sem rec* seminal receptacle, *test*, testes, *vit*, vitellaria, *uter*, uterus, *exc p*, excretory pore, *l c* Laurer's canal, *excr*, excretory duct, *ca*, caecum.

test

uter

l c

sem rec

excr

ov vit

2

3

4

gen p

sem ves

ov

l c

sem rec

test

vit

uter

5

excr. p

1

COCCIDIOSIS OF THE GUINEA PIG

BY

DORA PRIAULX HENRY

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CONTENTS

INTRODUCTION

In view of the large number of guinea pigs that are used for experimental purposes it is rather remarkable that so little is known of their parasites, including the coccidia. Labbé (1899) was apparently the first to notice coccidial oocysts in the intestinal contents of the guinea pig, which he believed were a variety of the coccidium of the rabbit. In the following year Strada and Traina (1900) reported coccidia in the intestinal contents of guinea pigs, associated with diarrhoea. They also found coccidia frequently in nodules on the liver.

In 1921 Bugge and Heinke described a rather extensive outbreak of coccidiosis in a colony of guinea pigs. They found 73 per cent of 180

animals infected with coccidia and stated that several animals which showed a high grade intestinal inflammation died. Cysts were seen only in the colon. Of 50 cysts measured the length varied from 15.9μ to 24.6μ, while the width varied from 12.2μ to 17.4μ. The average length was 20μ and the average width, 15μ to 16μ. Sporulation of the oocysts in Petri dishes at room temperature, with boric acid or thymol, began in two to three days and was completed in five to eight days. They concluded from their study that death was due to the inability of the guinea pigs to resist infection because of an insufficient diet.

Raebiger (1923), in a handbook on guinea pigs, refers to the occurrence of coccidiosis.

Dieben (1924) stated that the coccidium of guinea pigs could be distinguished from that of rats and was probably a separate species.

In the same year Sheather (1924) published an account of the life cycle of the coccidium of guinea pigs. He named this species *Eimeria caviae.* The developmental stages of this form were found only in the epithelium of the colon, especially in the upper portion and decreasing toward the rectum. Most of the animals experimentally infected were killed for the study of the life cycle, but he noted that one died from a fatal infection fourteen days after the initial feeding of oocysts. The hundred oocysts measured ranged in size from 17μ to 25μ in length by 13μ to 18μ in width.

Wenyon (1926) briefly reviewed the literature on the coccidia of the guinea pig.

LIFE CYCLE OF EIMERIA CAVIAE

In this study the oocysts of *Eimeria caviae* (pl. 11, figs. 1–9) encountered varied in length from 12.8μ to 25.6μ, and in width from 12.8μ to 22.4μ. This differs somewhat from the sizes given by Bugge and Heinke and by Sheather, in that oocysts smaller than those seen by any of these authors were found. Usually the cysts are oval in shape, although occasionally subspheroidal forms are encountered.

The oocyst wall is about 0.8μ thick, and is distinctly brownish in color in by far the greater proportion of the oocysts. The cytoplasm has a rather coarse appearance because of the large granules present. There is usually a relatively wide space between the cytoplasm and the cyst wall.

The sporulation process in *E. caviae* is similar to that found in other *Eimeria*. At first the rounded up cytoplasm of the cysts divides into four sporoblasts, which are more or less triangular in shape (pl. 11, fig. 2). The sporoblasts gradually lengthen and the edges become rounded (pl. 11, figs. 3 and 5); finally these are transformed into sporocysts, each of which contains two sporozoites and some rather granular residual material in the center (pl. 11, figs. 3, 6, 7, and 9). The sporocysts vary in length from 11.0μ to 13.0μ and in width from 6.4μ to 7.0μ.

For the study of the life cycle of *E. caviae* seven guinea pigs were killed at intervals of two days, beginning with the fourth day after infection. A study of sections from several regions of the colon of the animals killed on the fourth and sixth days failed to reveal stages in the life cycle. No doubt they would have been found if more material had been examined.

In sections of the colon of the guinea pig killed on the eighth day enormous numbers of merozoite cysts were found. In some regions every epithelial cell was invaded; the merozoite cysts were most commonly found in the glandular epithelium. A few isolated merozoite cysts are also found in later periods, i.e., in animals that died from coccidiosis. The size of the mature merozoite cyst (pl. 12, figs. 2, 5) varied from 10μ to 16μ. Sheather (1924) states that the maximum number of merozoites produced is thirty-two.

The first sexual forms were found on the tenth day; however, no animals were examined nine days after feeding. The younger forms, as well as the merozoite cysts, have been found beneath the nucleus of the host cell. The morphology of the macro- and microgametocyte (pl. 12, figs. 7 and 8) has been found to agree with that given by Sheather (1924). As a rule the microgametocytes varied from 13.0μ to 18μ in length; the maximum size, according to Sheather, is 25μ. The cytoplasm of the mature macrogamete differed from that of *E. tenella* and *E. maxima* in chickens in that the granules of stored material are not so large. Cysts have not been found before the eleventh day after feeding.

SPORULATION

Bugge and Heinke (1921) stated that the oocysts of the guinea pig began sporulation in two to three days and completed sporulation in five to eight days in boric acid or thymol. Sheather (1924) used a 5 per cent solution of bichromate of potash and found that sporulation had begun by the fourth day and the time necessary for complete sporulation ranged from five to eight days.

Throughout this work the method used has been that devised by Tyzzer (1929) for the sporulation of coccidia from fowls. In this procedure the faeces or intestinal contents containing the non-segmented oocysts were thoroughly mixed with 2 per cent potassium bichromate and incubated in Petri dishes. Using this method, sporulation had invariably begun in twenty-four hours and was completed in two to three days. *E. caviae* has also been found to sporulate quite readily in normal salt solution.

Sporulation time has been considered one of the several characteristics upon which the differentiation of species might be based. From the conflicting reports in the literature of the sporulation time of an identical species of coccidium it can be seen that if this characteristic is to be used as a criterion of species identical procedures must be followed.

Tyzzer (1928), in an attempt at standardization of methods for the determination of sporulation time, requires that a given temperature be used if comparable results are to be obtained. However, the control of temperature alone, when other factors such as the reaction of the suspension, relative quantities of fluid and solid, and gas tensions are ignored, may not give consistent findings. In order to differentiate definitely the sporulation time of two species it is highly desirable to have cysts of both species in the same container.

That the length of time necessary for the oocysts of a single species of coccidia to complete sporulation is a characteristic that is constant for that species and therefore of value in determining species has been amply shown by Tyzzer (1929) and Henry (1931a and b).

EXPERIMENTAL METHODS

Half of the animals used in the experimental work were raised in the animal colony of the Department of Zoology. Examinations for coccidia from time to time in this colony have always been negative. This can probably be explained by the fact that no new animals had

been brought into the colony for a year or longer. When it was necessary to bring in new animals for this study they were isolated for several days before they were admitted to the colony, during which time pooled samples of faeces were examined for coccidia.

In order that a deficient diet might not influence the course of the experimental infections, considerable attention was given to the matter of proper food. Throughout most of the work the animals received rolled barley and lawn clippings or lettuce daily. In a few of the preliminary experiments the guinea pigs used received green food only two or three times a week. However, uninfected animals were maintained in excellent condition on an identical diet.

To eliminate as far as possible the chance of cross infections great care was taken in handling both the normal and the infected animals. In order to insure thorough isolation three colonies were maintained in well separated rooms. In the first of these rooms only normal guinea pigs were kept. This group consisted of animals born in this colony and those added after quarantine and thorough examination. The care of these animals was delegated to a person who had no access to the infected group.

The second group consisted of animals experimentally or naturally infected and passing cysts. After recovered animals had been shown to be free of cysts by at least two examinations, using the sugar concentration method, they were removed to the third room, where they remained until re-infected.

To prevent, in so far as possible, the spread of infection from one animal to another in the inoculated group covered cracker tins were used as individual cages. At intervals of four or five days each guinea pig was removed to a sterilized can and the used can with its contents autoclaved.

The oocysts, either from faeces or intestinal contents, used in infecting guinea pigs were allowed to sporulate in the manner described above. When it was desired to infect animals the material containing the mature oocysts was strained through a fine wire gauze and thoroughly washed in normal salt solution. A pipette was used in giving the guinea pigs the infective dose. Although it is more time-consuming, this method is to be preferred to feeding the oocysts with milk or other food, as advocated by Andrews (1930). By the latter procedure it is not possible to be certain of the quantity of inoculum actually ingested by the test animal.

Except in a few cases, the number of cysts fed was not determined. However, the material to be fed was checked before feeding to insure the presence of a large number of sporulated cysts. Also, an attempt was made to feed all the animals in an experiment approximately the same number of mature oocysts. The routine was to feed the animals on three consecutive days. This was thought advisable, as it was then not necessary to force a large quantity on the animal at one time.

The sugar concentration method of Sheather (1923) was employed with success for the detection of small numbers of oocysts. A reliable concentration method is very useful, not only in the examination of animals whose history is not known, as in the case of purchased animals, but also in the detection of the first and the last oocysts passed by an infected animal.

Sheather's method consists of emulsifying the faeces with water, straining through wire gauze having thirty meshes to the linear inch, mixing the filtrate with an equal volume of a solution of sugar (specific gravity 1200), centrifuging for two minutes at 2000 to 2500 revolutions per minute, and examining the surface film for eggs or cysts.

PREPATENT PERIOD

The prepatent period as suggested and defined by Andrews (1926) is the lapse of time between the entrance of a parasite into the host and its appearance in the blood or faeces. This term is of particular value in reference to coccidial infections.

Sheather (1924) states that the first cysts of *E. caviae* may be found in the faeces of infected guinea pigs as early as the seventh day and as late as the thirteenth day after ingestion. Such variation in the length of the prepatent period is not in accordance with the more recent work on coccidial infections in general. In cases where the occurrence of accidental infections and the possibility of the presence of more than a single species have been reduced to a minimum, the prepatent period is usually found to be exceedingly constant. Under these conditions it is rare for the prepatent period of a given species to vary more than twelve to twenty-four hours (Andrews, 1926; Tyzzer, 1929, and Henry, 1931*b*). The possibility that factors other than those mentioned above may also influence the prepatent period will be considered in another section of this paper.

The twenty-two normal animals with no history of previous coccidial infections which were experimentally infected had prepatent

periods which varied from eleven to eleven and one-half days. In most of these animals the first oocysts were recovered almost exactly eleven and one-half days after the feeding of the sporulated oocysts. These results are in complete agreement with those reported in Tyzzer's monograph on the coccidia in gallinaceous birds, as well as with those reported later by Henry (1931b) for the same species.

In considering, first, the occurrence of oocysts earlier than the time designated here as the end of the prepatent period of *E. caviae*, the possibility that Sheather was working with more than one species seems improbable. According to the present author's interpretation of Sheather's report, the first cysts were detected in different animals at practically all times between the seventh and the thirteenth days after experimental inoculation. A possibility which cannot be completely ignored is that accidental infections occurring within one to three days just previous to experimental feeding may account for the early appearance of oocysts in the faeces. The passage of accidentally ingested oocysts through the digestive tract of experimental animals may also be a source of error in the determination of the prepatent period.

In the experiments designed to determine the prepatent period of *E. caviae* it was thought advisable from the author's earlier experience with the coccidia of chickens to carry out only one experiment at a time; i.e., animals were never fed infective material when other guinea pigs that were passing cysts were present. In this way the chance of the appearance of oocysts accidentally acquired was very greatly diminished. Also, it was possible to use guinea pigs from an isolated colony with no history of coccidial infection.

In the light of further work on this subject it is believed that the occurrence of oocysts later than the time here designated as the prepatent period of *E. caviae* can be adequately explained. This will be discussed later.

Under these conditions, the prepatent period of *E. caviae* in guinea pigs previously uninfected with this species is eleven to twelve days, usually eleven and one-half days.

COURSE OF THE INFECTION

The first oocysts to be recovered from an infected animal may be so few in number that a concentration method must be used in order to detect them. In most cases, however, sufficient oocysts are present eleven and one-half days after infection so as to be found in direct

smears from the faeces. By the end of the twelfth day large numbers of cysts are invariably found in animals fed the usual quantity of oocysts, and the number increases and reaches a maximum on the thirteenth day. Usually, no diminution in the number of oocysts is noted until the sixteenth day of the infection. If death does not occur, the oocysts gradually decrease in number until they are no longer found. The minimum length of time during which a heavily infected animal has been found to discharge oocysts is eighteen days. In animals other than those fed cysts over a long period of time the maximum length of the carrier state in the animals used was thirty-one days.

CLINICAL SYMPTOMS

The first symptom of infection noted in a guinea pig experimentally infected with *E. caviae* is the occurrence of diarrhoea. In all experimentally infected animals, with the exception of one, diarrhoea was a constant symptom. It was noticed for the first time in one animal as early as the tenth day after feeding cysts, and in another on the fifteenth day, but was more commonly found from the eleventh to thirteenth day (see table 1). In some animals (because of lighter infection?) diarrhoea lasted only one day, but usually it was found for four or five days. In general, it might be said that in those cases that ended fatally diarrhoea was more severe than in those cases that recovered. Also, with only one exception, in fatal cases diarrhoea was noted continuously from the day it first started until death.

After the first indications of diarrhoea are present, an occasional formed stool may be passed. However, on the following day only diarrhoic stools usually occur, and increased quantities of faecal material are discharged. This condition may continue, and usually does, until the death of the guinea pig, but occasionally toward the end of the life of the animal a string of very small pellets may be passed. In animals that recover from the infection diarrhoea may continue for as long a period and with the discharge of as large quantities of material as in animals that die of the infection. In two recovered guinea pigs diarrhoea lasted only one day, but in animals that died two days was the shortest period of diarrhoea.

Constipation was a rather constant symptom. This was particularly noticed about the eleventh and twelfth days, but rarely later. In the one guinea pig which showed no diarrhoea this condition was greatly aggravated; also, a marked loss of weight was noted (see

TABLE I

OCCURRENCE OF DIARRHOEA IN EXPERIMENTALLY INFECTED GUINEA PIGS

Number of guinea pig	Appearance of diarrhoea (Days after feeding cysts)	Disappearance of diarrhoea (Days after feeding cysts)	Termination of infection	Number of guinea pig	Appearance of diarrhoea (Days after feeding cysts)	Disappearance of diarrhoea (Days after feeding cysts)	Termination of infection
E6	12	15	D*	E50	13	15	D
E21	12	16	D	E75	13	16	R
E23	11	14	D	E76	13	16	R
E24	12	17	R*	E77	10	12	R
E28	15	15	R	E78	11	14	R
E33	12	14	R	E91	13	15	R
E34	12	13	D	E93	13	15	D
E35	11	13	D	E94	12	13	D
E36	11	15	R	E95	12	14	R
E47	....	....	R	E97	11	16	D
E48	12	13	R	E81	12	17	D
E49	14	14	R	E83	11	16	D

D*—died   R*—recovered

TABLE II

TEMPERATURES OF GUINEA PIGS FED OOCYSTS OF E. CAVIAE

Days after feeding oocysts	E33	E34	E35	E36
1	103.4	102.0	101.9	104.1
2	....	....	....	....
3	....	. .. .	....	.. ..
4	101.1	102.8	101.4	102.0
5	101.4	102.2	102.2	102.4
6	102.8	102.6	102.0	102.2
7	102.2	102.4	102.0	102.0
8	....	....	....	. ...
9	102.2	102.0	102.0	102.0
10	102.4	101.8	101.8	102.4
11	102.4	102.6	102.6	102.6
12	103.1	102.4	101.2	102.5
13	102.6	102.0	97.8 (died)	102.2
14	101.4	99.7		101.1
15	101.8	98.0		101.6
16	102.4	99.2		102.9
17	102.7	94.0 (died)		102.5
18	102.0			102.0
19	101.2			102.4
20	....			....
21	102.3			102.2
22	102.6			102.2
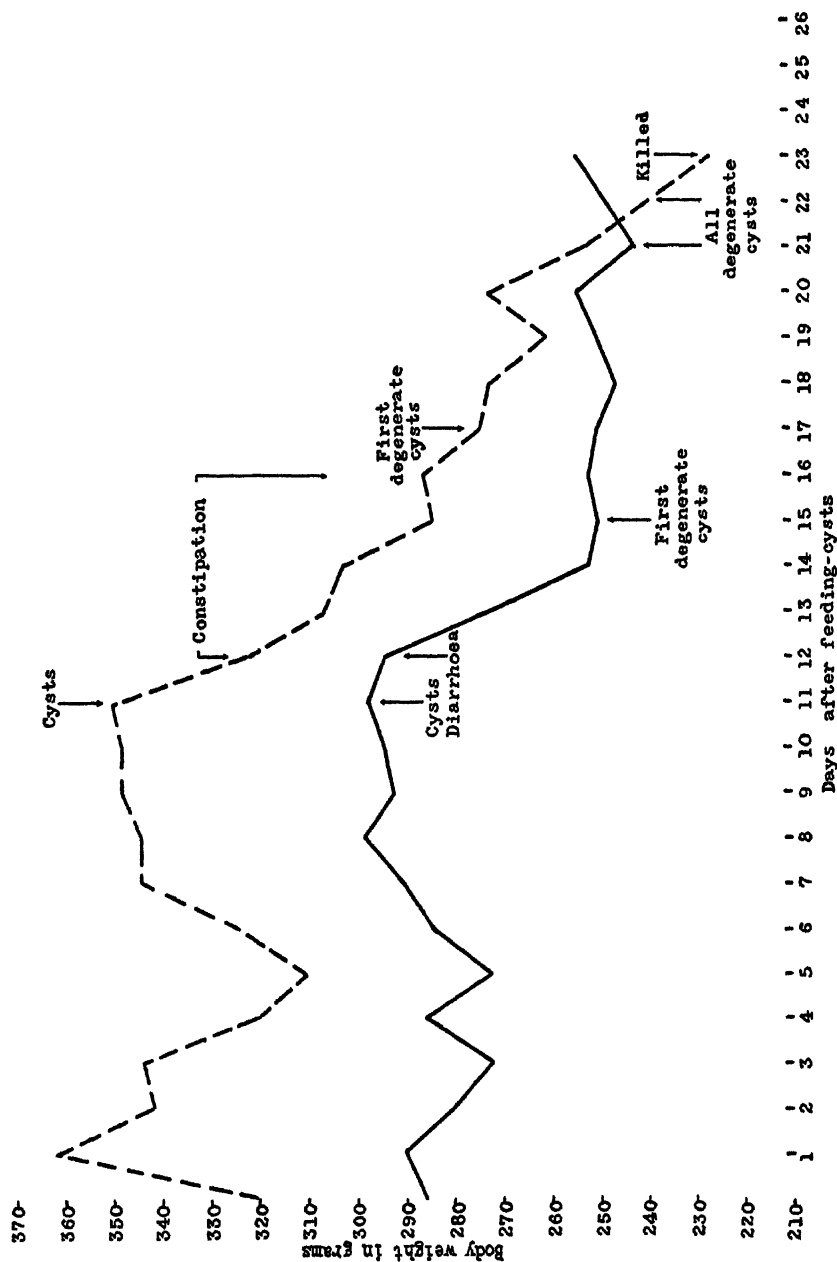
CHART 1

A curve of the daily weights of two experimentally infected guinea pigs (- - - - E 47, —— E 48)
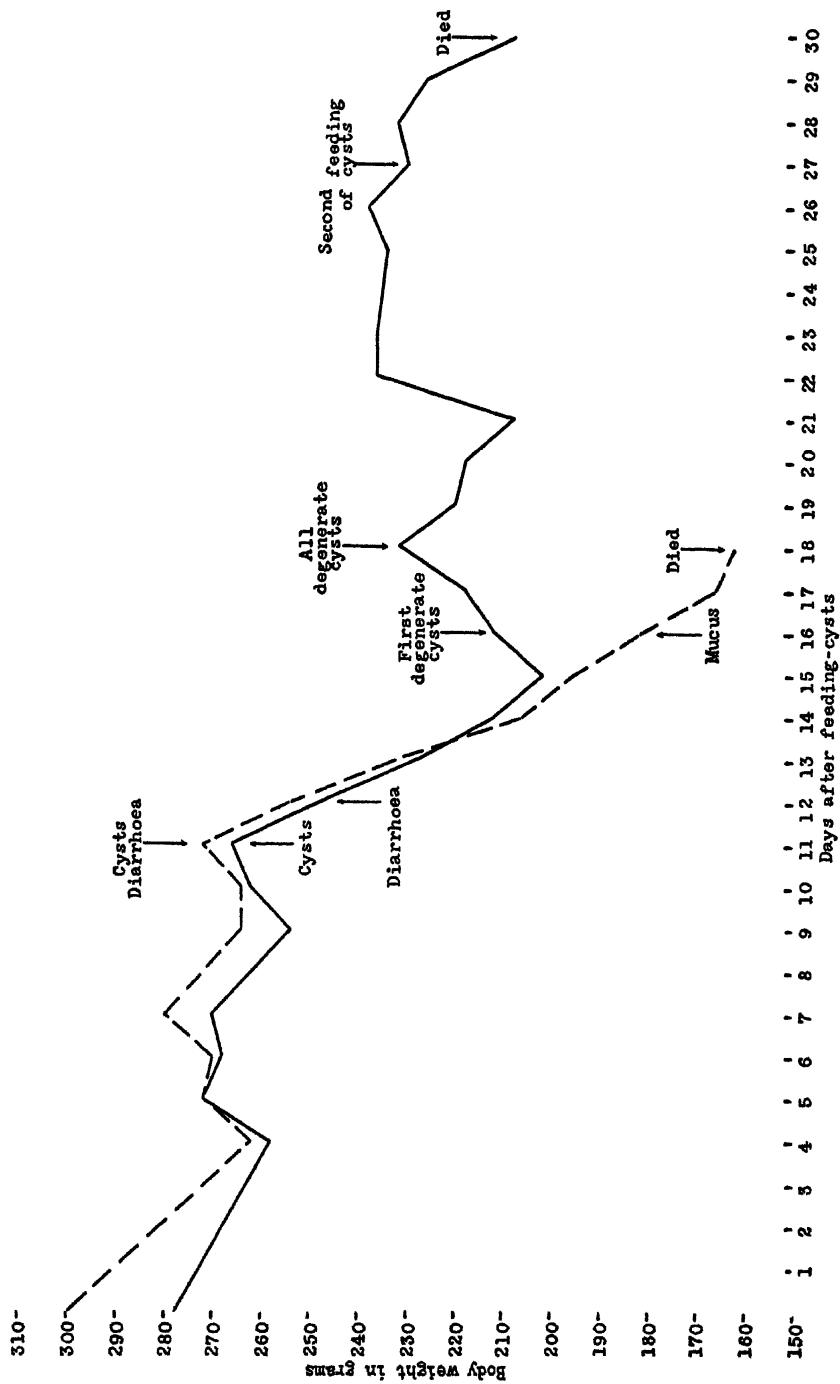
CHART 2

A curve of the daily weights of two experimentally infected guinea pigs; guinea pig E 34 (- - -) was a fatal infection and E 36 (———)

chart 1). In this case constipation was first noticed on the twelfth day after feeding; at this time no faeces were passed from 9:00 a.m. to 5:00 p.m. Constipation continued until the seventeenth day. This infection was also interesting as there were decidedly fewer cysts than in the other animals fed at the same time.

At about the time of the occurrence of diarrhoea the guinea pig becomes visibly ill. It is at first quiet, with hair ruffled and the body contracted in such a manner that the posterior portion of the animal is noticeably curved under the rest of the body. As a result of this position the animal appears much smaller than normally. A very marked loss of appetite was noticed in all animals and many took no food during the last day or so before death. Polyuria was noted in the sick animals.

Correlated with the appearance of diarrhoea was the striking drop in weight of the guinea pigs (see charts 1, 2, and 3). In some instances as much as 30 to 40 grams a day was lost, and in fatal infections in two cases there were losses amounting to almost half the body weights. No doubt diarrhoea, together with loss of appetite, is sufficient explanation for this drop in weight. In those animals that recovered from the infection, with the cessation of diarrhoea and with the decrease in number of oocysts passed, a steady gain in weight was shown. However, guinea pigs that had recovered from coccidiosis did not as a rule gain in weight as fast as normal animals.

Daily temperatures were taken on four animals infected with coccidia (see table 2). The asexual cycle of the coccidia appeared to have no effect whatsoever on the temperatures. In the two fatal cases the body temperature went down rapidly just before death. Guinea pig E34 showed a lower body temperature for four days previous to death than at any time in the preceding ten days, and lower than the body temperatures of either of the other three, with the exception of E35 just previous to death.

Often the discharges of infected animals contain varying amounts of mucus, which, when examined under the microscope, is found to consist chiefly of packed oocysts. The occurrence of mucus gives a most unfavorable prognosis. Possibly smaller amounts of mucus are passed in most heavy infections, but are not detected because of the nature of the discharges and the difficulty of examining all the faecal material. In guinea pigs that have died of coccidiosis large amounts of mucus are usually found inside the large intestine. Numerous immature forms of macrogametes are often seen imbedded in it.

In a few animals small amounts of blood were detected in the faecal specimens. Occasionally at autopsy blood-tinged contents of the large intestine were noted.

To summarize, the most constant symptom of coccidiosis in guinea pigs is diarrhoea. The guinea pigs appear visibly ill and there is a marked loss of weight. Often mucus, containing enormous numbers of oocysts, is noted, and less often blood-tinged discharges. Of the few animals tested, the body temperature showed a striking decrease at the time of death. All experimentally infected guinea pigs showed clinical symptoms of the disease.

PATHOLOGY

Bugge and Heinke (1921) described a high grade intestinal inflammation in guinea pigs infected with coccidia. They believed the animals were unable to resist the infection because of a faulty diet; however, the kind of food fed was not mentioned.

Sheather (1924) states that most of the experimentally infected guinea pigs used in his work were killed for the study of the life cycle, but one animal died fourteen days after feeding oocysts. He does not describe the autopsy findings in this animal.

In the present study thirty-two guinea pigs, twenty-four of which died of coccidiosis and six of which were killed when dying, were autopsied. Of this number thirteen were experimentally infected with coccidia. All of the animals showed clinical symptoms of coccidiosis described above, and all showed enormous numbers of oocysts in the contents of the large intestine.

Extreme emaciation was exhibited in all animals which died of the infection. In all cases the lungs, liver, and spleen were normal in appearance. No changes were noticed in the small intestine nor in the stomach, except that in the latter organ hemorrhagic areas in the mucous surface were encountered in a few instances. Microscopically, no indications were found that these gastric lesions were due to coccidial infection.

At autopsy the large intestine was frequently distended with gas and hyperemic. The walls were usually somewhat thicker than normal and the patches of lymphoid tissue along the entire length of the colon were exceedingly prominent. Petechia on the mucosa were invariably found and occasionally larger hemorrhagic areas were noted. The small hemorrhages were the most striking macroscopic lesions found.

In some cases the entire inner surface of the anterior portion of the colon was studded with these pinpoint hemorrhages. The petechia occur throughout the length of the colon, but usually were not as numerous in the rectum as in the anterior portion. The contents consisted of mucus, in varying amounts, which contained masses of oocysts. Great numbers of oocysts were found in the contents in all animals examined. Often cysts were seen which were surrounded by the remains of epithelial cells. Commonly, immature forms of the parasite were noted in the invaded cells. In some cases minute amounts of blood were present. Frequently, small white or yellowish-white placques were seen, especially in the proximal half of the colon. In experimentally infected animals these were found only in those animals which were sick for a comparatively long period (five or six days).

In addition to the hemorrhages described, marked oedema was observed. In a few of the animals autopsied the mesentery associated with the loop of the colon five to six inches below the ileo-caecal valve showed gelatinous infiltration and there were marked oedema and enormous numbers of oocysts in the large intestine in this region.

The lymphoid tissue of the caecum stood out prominently; often small hemorrhagic areas were present on the mucosa, particularly near the ileo-caecal valve. The blood vessels were injected. Frequently the caecum, as well as the colon, was distended with gas. Usually many coccidia were present in the lumen.

The mesenteric lymph nodes always showed enlargement and usually were hemorrhagic. This was particularly noticed in those nodes draining the ileo-caecal region.

MICROSCOPIC PATHOLOGY

As previously mentioned, no stages of coccidia were found in sections of the lesions found in the stomachs of animals experimentally and naturally infected with coccidiosis.

In the small intestine a few isolated coccidia were seen; no lesions were noted.

The caecae of three animals, two naturally infected and one experimentally infected, showed some evidence of invasion by the coccidia. In one case large areas had been invaded and practically every epithelial cell contained coccidia in some stage of development.

The colon, both in experimentally and in naturally infected guinea pigs, showed extensive invasion of the epithelial cells by the coccidia

(pl. 13, figs. 1–3, pl. 14, figs. 1–2), extending into the crypts of Lieberkühn. In many places the whole mucosa had been lost; the epithelial cells had been destroyed, leaving large spaces containing some cellular debris and coccidia. Ruptured capillaries, with resulting accumulation of red blood cells, were observed in many cases (see pl. 13, figs. 2 and 3). These usually occur just underneath the cuticle. Small cell infiltration was noted wherever there were considerable numbers of coccidia.

Sections cut through the white placques discussed above reveal large numbers of coccidial oocysts (see pl. 14, figs. 3 and 4) accumulated in masses measuring as much as 150–200μ in diameter. The color of the placques varies from grayish to yellowish-white or white, according to the degree of maturity of the coccidia. In some cases there is a suggestion that encapsulation of the accumulated oocysts has occurred by proliferation of the connective tissue. This has been described by Theobold Smith (1910) in an adult rabbit, and he has suggested that this might be one means of disposing of the parasite, leading to a relative immunity. The phenomenon just described in the guinea pig may be comparable to that described by Smith, but its rôle in immunity has not been investigated. Somewhat similar lesions have been described by Tyzzer (1929) in *E. acervulina* infections in the chicken.

MORBIDITY AND MORTALITY

In the twenty-two guinea pigs raised in the colony in which no coccidiosis had occurred for at least one year, experimental infections always resulted in the clinical symptoms described above, namely, visible illness, loss of appetite, marked decrease in weight, lessened activity, and diarrhoea.

The age of the animals apparently did not influence the course of infection, nor was any difference in susceptibility noted between the sexes. In one instance an eight- or nine-months-old guinea pig succumbed to an experimental infection. Other experimental animals ranged in weight from 250 to 450 grams. The relation of age to severity of infection will be discussed more fully under immunity.

A mortality rate of 40.9 per cent was established for *E. caviae* by the death of nine of twenty-two animals infected. Tyzzer (1929) found a mortality of approximately 83 per cent in experimentally induced *E. tenella* infections in chickens. This species is the most

pathogenic of the coccidia occurring in chickens. Chapman (1929), working with rabbit coccidia, found 100 per cent mortality in experimental infections of coccidia-free rabbits when 100,000 or more sporulated oocysts were fed. However, her results are not comparable to those found in the guinea pig and the chick, as the coccidia-free rabbits may have been more susceptible as a result of the conditions under which they were raised—taken from the litter at ten or eleven days and fed sterilized food. Nevertheless, it may be said that the mortality rate of experimentally infected guinea pigs is not so high as in *E. tenella* infections in chickens, nor intestinal coccidial infections in rabbits.

The following table shows the day of death of the experimental infections with *E. caviae*.

TABLE III

MORTALITY IN E. CAVIAE INFECTIONS

Death (days after infection)		Number of guinea pigs
13	....	2
14	....	1
15	....	1
16	....	2
17	....	1
18	....	1
25	....	1

IMMUNITY

Until recently there has been a great difference of opinion concerning the occurrence of immunity in coccidial infections. One of the primary reasons for this has been the failure to recognize the multiplicity of species in a single host. Because of this failure various workers studying coccidiosis in the same animal were able to obtain dissimilar results. Hadley (1911) found no evidence of immunity or resistance to coccidial infection in chickens, whereas Beach and Corl (1925) noted that chickens recovering from coccidiosis showed a resistance to subsequent infection. Johnson (1927) found that a high degree of resistance was regularly developed by experimental inoculation of coccidia in chickens. He also noted that commercially reared fowls usually showed a definite resistance to coccidial infection, while carefully reared cage fowls were markedly susceptible. Johnson

(1927) recognized two types of coccidia, the caecal and intestinal forms. Young (1929), also working with the coccidia of chickens, found no evidence of immunity or resistance. However, he recognized only one species of coccidia. But it was not until Tyzzer (1929) differentiated four species of coccidia in chickens that true knowledge of immunity could be sought in these animals. In brief, Tyzzer found that chickens experimentally infected with one species, i.e., *E. tenella,* exhibited an immunity to subsequent infection with this species, but no immunity to subsequent infection with *E. maxima, E. acervulina* or *E. mitis.* This was later confirmed by Henry (1931*b*). The conflicting reports on immunity to coccidial infections can be attributed to this failure to recognize the species present.

Andrews (1926) found that one infection of *Isospora felis* renders dogs and cats non-susceptible to a second exposure and that this immunity lasts for at least seven months and probably for life. He believes it probable that the mechanism of immunity operates in a manner similar to host-parasite specificity.

Chapman (1929), working with rabbits, found some evidence of a relative immunity gained by a light infection with coccidia, though she was unable to demonstrate the presence of antibodies in the circulating blood. As rabbit coccidiosis is chronic in nature, it is possible that it is not comparable to coccidiosis in birds and in such mammals as dogs and cats and guinea pigs; however, further light will no doubt be shed on the occurrence of immunity in rabbits when pure infections with the five species differentiated by Kessel (1929) have been obtained. Chapman was not working with *E. stiedae,* which is limited to the liver, but the possibility cannot be ruled out that mixed infections with the intestinal forms were present.

Other workers with the coccidia of the above and other animals have found evidence for and against the production of immunity by coccidia. In many cases these conclusions were not the result of experimentation but merely an attempt to explain the often observed resistance of older animals to infection.

The Occurrence of Degenerate Cysts

Contrary to the conditions found in rabbits, coccidiosis in guinea pigs is a self-limiting infection. In the first group of guinea pigs experimentally infected with *E. caviae* a very striking thing was noticed. (See Henry, 1931*c*.) If the infection did not prove to be fatal, diarrhoea ceased and the animal usually began to gain in weight;

in other words, recovery from the disease was in full progress. At about this same time an examination of the faeces showed numerous oocysts, though fewer in number than at the height of the infection; but in addition to the ordinary normal non-segmented oocysts, a varying number of obviously degenerate oocysts were seen. The following day a greater proportion of degenerate over normal cysts was found, and by the third to fifth day after their first appearance almost all of the cysts were degenerate (see charts 1–3).

A few days later no normal cysts were found, but degenerate cysts in decreasing numbers were passed for several weeks after feeding sporulated cysts. This phenomenon is very regular in occurrence and no experimentally infected guinea pig failed to show degenerate cysts (see table 4). They have also been found in all animals examined which recovered from a natural infection.

The most marked morphological change in the degenerate cysts (pl. 12, figs. 1, 3, 4, 6) is shown by the contents. Instead of cytoplasm composed of rather regular small granules and the nucleus usually clearly visible in the center, the granular cytoplasm appears as an amorphous mass or is absent and in its place varying numbers of comparatively large globules are found. Often no evidence of cytoplasm is found, the cyst appearing to be empty except for the globules, which are frequently concentrated on one side of the cyst (pl. 12, fig. 1). The greater number of degenerate cysts exhibit a form in close agreement with that described above. Occasionally, other types of degenerate cysts are found. In some instances the globular material is not seen and the cytoplasm is heavy and extends to the inner surface of the cyst wall (pl. 12, fig. 6). These cysts are small and appear shrunken and misshapen.

In some cases the cyst wall is intact, but often rifts in the wall can be detected (pl. 12, fig. 4). Cysts with injured walls are more rarely encountered in the first few days of their passage than later. The wall of the degenerate cyst quite commonly has extraneous material adherent to its outer surface (pl. 12, fig. 1).

Although normal cysts of *E. caviae* are occasionally seen inside epithelial cells or with bits of cellular debris attached to the cyst wall, this is by no means common as compared to the occurrence in degenerate cysts.

No reference concerning degenerate cysts has been found in the literature on coccidiosis except in that of Chapman (1929) on rabbit coccidia. She states that an experimentally infected animal "showed many oocysts in the faeces on the 16th, several on the 19th, a few

TABLE IV

Results of Initial Coccidial Infections of Guinea Pigs

No. of guinea pigs	First cysts	First degenerate cysts	Last normal cysts	Died	Last cysts	Remarks
E6	11	none	16	16	....	
E21	11½	none	16	16	....	
E23	11½	none	14	14	: ..	
E24	11½	19	22	....	28	
E28	11½	18	19	....	28	
E33	11½	17	19	....	48	Possibly ingested from infected animals. (Normal cysts appeared on the 30th-35th days.)
E34	11½	none	17	17	....	
E35	11	none	13	13	: ..	
E36	11	16	18	....	27	Fed cysts on 27th day.
E47	11	17	19	: ..	23	Killed 23rd day.
E48	11	15	18	....	23	Fed cysts on 23rd day.
E49	11½	16	17	: ..	17	Fed cysts on 17th day.
E50	no specimen	16	18	18	....	
E75	11½	15	20	....	21	Fed cysts on 21st day.
E76	11½	14	19	....	44	Fed cysts 8 consecutive days.
E77	11½	14	18	: ..	26	
E78	11½	14	20	: ..	31	
E94	11½	none	13	13	: ..	
E95	11½	15	: ..	: ..	: ..	
E97	11	16	17	: :	: ..	

In each case the number indicates the days after feeding cysts.

degenerated ones on the 21st, and none thereafter." As this is the only reference in her paper to degenerated cysts, no conclusion can be reached at this time as to whether these forms were comparable to the forms found in the guinea pig.

In a few instances the author has seen degenerate cysts in the caecal contents of swine obtained from the slaughterhouse, but from the data at hand it cannot be said with any degree of assurance that these represent the same phenomenon found in the guinea pig. It is well known that even when oocysts are allowed to sporulate in the best possible environment, some of the oocysts fail to mature and these often exhibit a very abnormal appearance. For this reason it is possible that the degenerate cysts seen in swine were ingested; possibly abnormal cysts that had failed to sporulate for one reason or another.

In order to understand this phenomenon it is necessary to understand the factors that bring about the change from the asexual cycle to the sexual cycle, which results in the production and subsequently in the elimination of oocysts. There are many theories as to the causes responsible for this change, but they have no experimental evidence to support them. It seems clear that the production of asexual forms calls forth some definite response of the host and as a result of this the sexual cycle begins, which, from the standpoint of the parasite, provides for dissemination and propagation of the species, but which tends to rid the host of its parasite.

In the guinea pig the production or occurrence of oocysts may call forth another response from an immune or partially immunized host, resulting in the formation of degenerate cysts. It seems more reasonable to suppose, considering the structure of the oocyst wall, that the degenerate cyst is not the result of the influence of this factor or factors on a perfectly formed oocyst, but rather upon an earlier stage (macro- or microgamete, or both).

RE-INOCULATION

In order to determine whether or not guinea pigs which had recovered from coccidiosis showed any resistance to subsequent infection, animals which had recovered from one infection were fed oocysts at various intervals after the first feeding (see table 5). One or more normal animals were included in each experiment for comparison. In all cases these control animals showed oocysts on the eleventh day, diarrhoea at some period, loss of weight, etc., as described above.

TABLE V

RESULTS OF RE-INOCULATION OF GUINEA PIGS RECOVERED FROM AN INITIAL EXPERIMENTAL COCCIDIAL INFECTION

No. of guinea pig	Last cysts	Days after first feeding of cysts	Appearance of cysts	Type of cysts first passed	Symptoms	Last cysts	Remarks
E24	28 degen.	45					Died 10th day. Few degenerate cysts.
E28	28 degen.	45	12	degen. no N	Loss in weight	21	Fed cysts again 21st day.
E36	27 degen.	27					Died 2nd day. N cysts (fed?).
E33	49 possibly re-infected from others in same room	49	15	N	Slight loss in weight	24	Fed more cysts 24th day.
E48	23 degen.	23	12	1 degen.	diarrhoea 13th day N cysts	17	Fed more cysts 17th day.
E49	17 N and degen.	17					No cysts found.
E75	21 N and degen.	21	10	degen. and N	slight diarrhoea 13th day	24	Fourth day few degenerate. Eighth day negative. Eleventh day few cysts, degenerate and normal. Twelfth day few cysts, mostly normal. Thirteenth day few cysts, mostly normal. Fourteenth day few cysts, one-half normal. Fifteenth day to twenty-first, only degenerate. Twenty-fourth day few cysts, normal and degenerate. Twenty-fifth day negative.

In each case the number indicates the days after feeding cysts.
N=normal cysts; degen. =degenerate cysts.
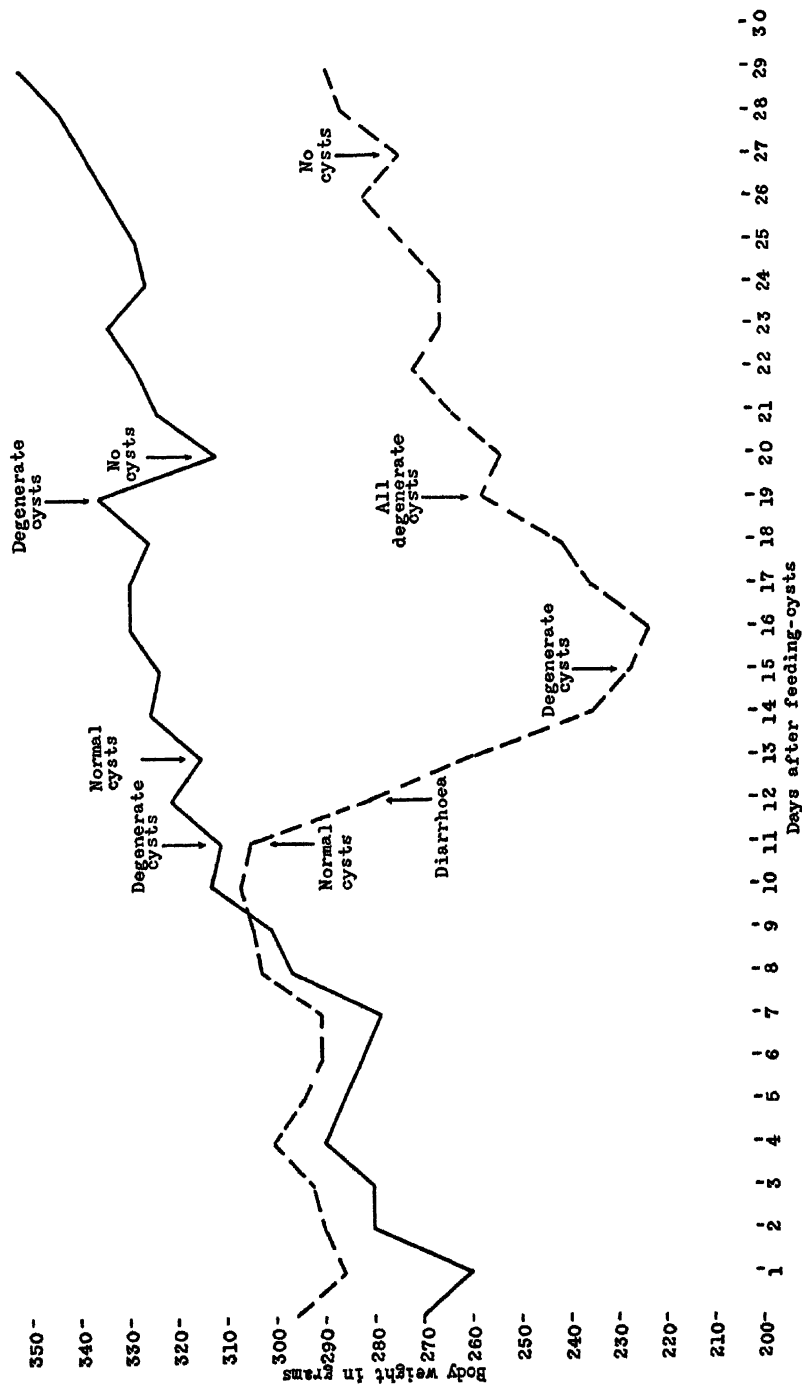
CHART 3

A curve of the weights of two experimentally infected guinea pigs. Guinea pig E77 (- - -) had an initial infection and guinea pig E67 (——), a secondary infection. The latter animal showed a steady increase in weight, few cysts, and no diarrhoea.

As the results in table 5 show, two of the seven previously infected animals died before the end of the prepatent period; this will be discussed below. Of the other five animals, one showed diarrhoea for a single day (13th) and another showed slight diarrhoea the same day. The first of these two guinea pigs (E48) was inoculated with oocysts on the 23rd day after the first feeding and was passing degenerate cysts at the time of re-inoculation. The other (E75) was inoculated earlier in relation to its primary infection (21st day) and was passing both normal and degenerate cysts at the time of re-inoculation. Two of the other three animals showed some loss of weight following re-inoculation; one showed no symptoms. Although there were some variations in the symptoms shown in the re-inoculated animals, in no case did they approach in severity those found in initial infections (see table 1).

Using the criterion of cyst formation as evidence of infection, one animal showed complete resistance to re-inoculation. The remaining four exhibited an altered prepatent period, i.e., in three cases, a delayed period, and in one (E75) an accelerated one. This is very striking in comparison with what is found in initial infections, i.e., in twenty-two animals cysts were found eleven to eleven and one-half days after feeding cysts (see table 4).

In addition to the lack or lessened severity of the clinical symptoms, the altered prepatent period, and in one case complete resistance exhibited by re-inoculated guinea pigs, the occurrence of degenerate cysts either before or accompanied by normal cysts is commonly noted (see chart 3). All of these factors considered together seem to warrant the conclusions (a) that coccidial infections cause reactions and responses on the part of the host which may morphologically and physiologically alter the parasite; (b) that no correlation between the various expressions of this response and time of re-inoculation is possible from the experiments so far performed. It seems probable that the extent of the primary infection, its duration, and the individuality of the host may all influence this relation.

In this connection it is interesting to consider the findings of Andrews (1926) in *Isospora felis* infections in cats. In younger animals, weighing 750 grams or less, the prepatent period was five or six days; in two older kittens, weighing more than 1750 grams, the prepatent period was four and three days, respectively. Andrews states that age may be a factor which influences the prepatent period, although he considers the possibility that the oocysts found may be some

of the originally inoculated oocysts. In later experiments in which he re-inoculated cats and dogs which had recovered from an infection he was unable to reinfect these animals. In considering the mechanism by which this immunity operates, Andrews suggests two possibilities, first, that the multiplicative cycle continues at low ebb, producing few or no oocysts. This, he states, is supported somewhat by the fact that he found at rare intervals in immune animals a solitary oocyst (not recorded in his protocols, as he does not believe ''such findings constitute conclusive evidence of coccidial infection''). However, since oocysts were never found more than ten days after re-inoculation, he is led to believe that they are probably oocysts originally inoculated whose passage through the intestine has been delayed. A second possibility is that the test inoculation produces an aberrant, mild type of infection and few oocysts are produced.

In the light of what has been found in the guinea pig, it seems possible that the condition found in cats and dogs may be comparable, although a greater resistance to reinfection is shown in the latter animals. There is also the possibility that the shorter prepatent period shown by the older cats might be an indication of immunity derived from a previous infection. In the guinea pig, as will be shown later, a delayed rather than an accelerated prepatent period occurs more commonly.

In a later experiment (see table 6) seven guinea pigs which survived a natural infection involving a group of eleven animals were re-inoculated with cysts twelve days after the death of the other four animals. Large numbers of cysts had been found in the faeces of all seven animals during the course of the first infection. No cysts were found by direct smear method in the faeces of these animals for two days previous to re-inoculation, but all had cysts four days previous to reinfection. In six, cysts were found on the eleventh day, and in one, on the tenth day. All animals except one (E70) showed an extremely light infection, which was characterized by the occurrence of degenerate cysts on the eleventh day or very soon thereafter. The guinea pig (E64) which showed a foreshortened prepatent period had been extremely sick as a result of the previous infection; in the second infection few cysts were noticed, only a few normal cysts on the thirteenth day and no cysts thereafter. The heaviest infection, as evidenced by the number of cysts passed, was seen in guinea pig E70, but no symptoms were noticed; in fact, there was a steady increase in weight. Cysts were found for a somewhat shorter period than in the

former experiment. The results of feeding oocysts a second time to guinea pigs are in sharp contrast to what is found in primary infections. For comparison, the results obtained from a control animal are included in table 6, together with those of seven previously infected guinea pigs. E78 received a light infecting dose in comparison with that fed the other animals in this group. In the control animal the first cysts were found on the eleventh day, the first degenerate, on the fourteenth day. Diarrhoea on the eleventh to fourteenth days and an extreme loss of weight were noticed. In addition, cysts were found until the thirty-first.day, a decidedly longer period than in either of the two groups of re-inoculated animals.

Seven animals purchased from a dealer were found to be passing a medium number of oocysts on arrival (examination of composite faecal samples). About two months later five of these guinea pigs were fed cysts (see table 7). One died two days later; in the remaining four the first cysts were found on the thirteenth day in three animals, and on the fourteenth day in one. In all cases the first cysts passed were normal ones and degenerate cysts were not found until a somewhat later time than in the two previous groups. In two cases symptoms of coccidiosis were noted; E62 showed diarrhoea on the fourteenth and fifteenth days and many cysts, E63 showed slight loss of weight, but very few cysts, and only on the fourteenth and fifteenth days. No symptoms were observed in the other two animals of this reinfected group.

Of the seven animals originally obtained from the dealer, the two omitted from the first reinfection group received inoculum six days later from a different source from that given the first five animals. In one, cysts were first noted on the thirteenth day, in the other on the fourteenth; both showed diarrhoea. The first degenerate cysts were found on the fifteenth day. The results obtained from a normal animal, E75, inoculated at the same time and with the same dose, are included in table 7, together with the results of the two re-inoculated groups just discussed.

By a comparison with table 6 it will be seen that those animals included in table 7 are characterized by a longer prepatent period, symptoms of coccidiosis in several animals, and lack of degenerate cysts at the onset of the infection. That this difference can be accounted for by the heavier initial infection and the shorter interval between inoculations of the group in table 6 seems probable but cannot be definitely decided from the data at hand.

TABLE VI

RESULTS OF RE-INOCULATION OF GUINEA PIGS RECOVERED FROM A NATURAL COCCIDIAL INFECTION

No. of guinea pig	History	Appearance of cysts	Type of cysts first passed	Symptoms	Last cysts	Remarks
E64	Received 9-XII-30 from dealer; 22-XII-30 4 of same lot died of coccidiosis; 8-I-31 fed cysts dose No. 7	10	2 degen.		13	Only cysts seen 2 degenerate on 10th day, 2 degenerate on 11th, none on twelfth, and few degenerate and 2 normal, on thirteenth.
E65	"	11	N		16	First degenerate on 13th day. Slight infection (fed 8 doses of cysts).
E66	"	11	N		16	First degenerate on 12th day; no normal seen after 12th day; very slight infection.
E67	"	11	very few degen.		19	Thirteenth day few normal cysts. No normal cysts after 14th day.
E68	"	11	few normal		19	First degenerate 14th day. No normal after 15th day.
E69	"	11	degen. and few normal		15	No cysts on 12th day; 1 normal seen on thirteenth; few on 14th, many degenerate; 15th 5 normal and 2 degenerate only cysts seen.
E70	"	11	normal		21	First degenerate on 13th day; last normal on 16th, more cysts than in any of the preceding animals; fed on the twenty-first day.
E78	normal animal 1st time fed; fed approx. 300,000 cysts. Small dose compared to massive dose fed above	11	normal	diarrhoea 11th-14th day—extreme loss of weight	31	First degenerate on 14th day; no normal after 20th day.

In each case the number indicates the days after feeding cysts.

TABLE VII

RESULTS OF RE-INOCULATION OF GUINEA PIGS RECOVERED FROM A NATURAL COCCIDIAL INFECTION

No. of guinea pig	History	Appearance of cysts	Type of cysts first passed	Symptoms	Last cysts	Remarks
E59	Purchased; coccidia found, no diarrhoea; no deaths after arrival	13	normal		25	First degenerate cysts 16th. Few cysts.
E60	"	13	4 normal		19	First degenerate cysts 16th. Few cysts.
E61	fed 31-XII-30 dose No. 7	13				Died 2nd day. No cysts found.
E62	fed 31-XII-30 dose No. 7	13	normal	diarrhoea 14th-15th day	20	First degenerate 18th day.
E63	fed 31-XII-30 dose No. 7	14	few normal	slight loss in weight didn't eat all of food	15	Very slight infection; 15th, very few cysts, none thereafter.
E72	fed 6-I-31 dose No. 8	13	few normal	diarrhoea 15th-16th day	18	First degenerate 15th day.
E73	fed 6-I-31 dose No. 8	14	medium no. of normal	slight diarrhoea 13th day slight loss of weight	18	First degenerate 15th day; died 27th day.
E75	normal animal 1st infection fed 6-I-31 dose No. 8	11	normal	diarrhoea 12th-14th day	21	First degenerate 15th day. Twenty-first day normal and degenerate; re-inoculated.

In each case the number indicates the days after feeding cysts.

Another group of twelve animals, approximately six weeks old, was obtained from a dealer. The first and second days after arrival all animals were negative for coccidia. They were fed oocysts on the second day after arrival. From the results obtained (see table 8) it would seem very probable that at least ten of these animals had been previously infected. One animal died on the ninth day. In two cases, E81 and E83, an extremely heavy infection resulted, with diarrhoea for four and five days, respectively, and great loss in weight. In the remaining nine guinea pigs there were slight infections; in three, E79, E86 and E90, extremely light infections resulted. Only one cyst, a degenerate one, was found in guinea pig E86.

Another interesting thing about these animals which throws some light on their previous history is the finding of a nematode worm in the caecal contents, or eggs in the faeces of most of them. This worm has been identified through the kindness of Mr. O. L. Williams as *Paraspidodera uncinata* (Rudolphi, 1819) Travassos, 1914. Worms in the caecal contents or eggs in the faeces of the animals still living have been found in all but two of the twelve animals, namely, E81 and E83. These animals were the only ones which showed heavy infections with coccidia and symptoms of coccidiosis. Therefore it seems very likely that these two animals may have come from a different pen or cage than the other ten purchased. This is the only instance in which nematodes have been found in the guinea pigs used for this study, although the pigs had been obtained from a number of different sources. The nematode infection did not spread among the experimental animals, except in the case of one control animal kept in contact with E79 and E90 for nine days. Larval forms of this nematode were found in the caecal contents of this guinea pig. Three other guinea pigs kept for the same length of time with the animals infected with nematodes did not become infected.

The effect of more than two inoculations, the results of which are shown in table 9, does not differ greatly from that of two infections. Two guinea pigs died three days after the third inoculation, one died on the eighth day, and one died on the ninth day after the third inoculation. One died two days after the fifth inoculation. Of the other animals, one (E59) showed no cysts up to and including the fourteenth day, one (E33) showed no cysts up to and including the seventeenth day, two (E70 and E68) passed cysts on the tenth and fifth days, respectively, after inoculation. In these last mentioned animals degenerate cysts were found in the faeces of E70 on the day of re-

TABLE VIII

RESULTS OF INOCULATION OF GUINEA PIGS WITH UNKNOWN HISTORY

No. of guinea pig	History	Appearance of cysts	Type of cysts first passed	Symptoms	Last cysts	Remarks
E79		16	1 normal		16	Only one cyst seen.
E80		14	3 normal		21	First degenerate 16th day, very few normal.
E81	Negative for coccidia at time of purchase	11	normal	diarrhoea 14th–17th days; loss in weight		Died 34th day, degenerate cysts only.
E82	Approximately 6 weeks old	11	normal		25	Very slight infection. Degenerate first on 16th day.
E83	"	11	normal	diarrhoea 12–16 days loss in weight	27	Fed cysts 27th day, very few degenerate.
E84	"					Died ninth day.
E85	"	14	normal	loss in weight	22	First degenerate 17th day. Killed 22nd day— few degenerate. Pneumonia.
E86	"	12	1 degen.		12	Only cyst seen 1 degenerate on 12th day.
E87	"	14			21	First degenerate 15th day, few cysts.
E88	"	12	normal		37	First degenerate 15th day; medium number of cysts on 12th–14th days.
E89	"	11	1 normal		25	First degenerate 16th day; few cysts.
E90	"	14	2 degen.		17	Very few normal cysts. Fed cysts on 17th day (degenerate and few normal)

In each case the number indicates the days after feeding cysts.

TABLE IX

RESULTS OF TWO OR MORE RE-INOCULATIONS OF GUINEA PIGS RECOVERED FROM EXPERIMENTAL AND NATURAL INFECTIONS

No.	No. times previously fed cysts	Days after feeding		First cysts	Type of cysts first passed	Symptoms	Last cysts	Remarks
		First	Second					
E28	2	67	23					Died third day.
E48	4 2nd	40	17 (1 degen.)					Fifth day fed.
	3rd	45	24 (1 degen.)					No cysts.
	4th	approx. 4 months	about 2 months					Died 3rd day.
E33	2	72	24					Degenerate cysts when fed. No cysts found up to 17th day.
E59	2*	?	27					No cysts found up to 14th day.
E63	2*	?	27	11	1 degen.			1 degenerate on 11th day. 1 degenerate on 13th day only cyst seen.
E70	2*	?	21	10	1 normal	mucus 14th day—many cysts	21	Degenerate cysts when fed. Few cysts until 14th day. Few cysts 15th and 16th days. No normal after 17th day.
E72	2*	?	21					Few degenerate cysts when fed. Died 8th day.
E79	2*	?	17	14	few normal few degen.			No cysts when fed. Few cysts 15th and 16th days. Died 17th day.
E90	2*	?	17	11	few degen.			Normal and degenerate when fed. First 2 degenerate on 12th day. No normal. 14th and 15th few normal, also degenerate. 16th, 17th, 18th only degenerate. Killed.

TABLE IX—*Concluded*

RESULTS OF TWO OR MORE RE-INOCULATIONS OF GUINEA PIGS RECOVERED FROM EXPERIMENTAL AND NATURAL INFECTIONS

No.	No. times previously fed cysts	Days after feeding		First cysts	Type of cysts first passed	Symptoms	Last cysts	Remarks
		First	Second					
E60	2*	?	37	11	1 degen.		19	12th only 1 degenerate seen. 13th-15th very few cysts, degenerate and normal. 16th-17th few normal cysts. 18th-19th degenerate cysts.
E68	2*	?	31	5 not examined 6-10 10	2 degen. 4 degen.	.	19	Negative when fed. 11th day 1 degenerate. 13th no cysts. 14th-15th 1 normal. 16th none. 17th 1 normal. 18th none. 19th 1 normal.
E39	2*	?	31	14	1 normal		18	15th day 1 degenerate. 16th-17th few normal. 18th 1 degenerate.
E80	2*	?	27			quiet, sick second day		(Last cysts before feeding 21st day). Died 3rd day.
E83	2*	?	27					Few degenerate cysts 27th day. 6th day few degenerate. 8th day died; medium number degenerate cysts.
E59	2*	?	41	14	few degen.; few normal		16	16th 1 degenerate only cyst seen.
E84	2*	?	32	16	1 normal		16	
E82	2*	?	31	13	1 degen.		17	14th 1 degenerate, 15th 1 degenerate, few normal. 17th 1 degenerate.
E89	2*	?	31	11	2 degen.		17	12th 1 degenerate, 13th negative. 15th 1 normal? 2 degenerate. 17th very few degenerate. Not examined later.

* First infection was natural.   Number in each case indicates days after feeding.

inoculation, but eleven days had elapsed since any cysts had been found in E68. In the remaining nine re-inoculated animals degenerate cysts only were found on the eleventh day in four animals, and on the thirteenth day in one animal; three animals passed cysts, for the first time during the current inoculation, on the fourteenth day. Of these, two showed both normal and degenerate cysts, and the third had only normal forms. In one animal the first cysts were found on the sixteenth day. Symptoms were noted in only one guinea pig, E70, in this group. The previous history (see table 6) of this animal was very interesting, as it belonged to a lot which was known to have had a heavy natural infection and was fed cysts a short time after the last cysts of this infection were found. Normal cysts were noted on the eleventh day, the first degenerate ones on the thirteenth, the last normal on the sixteenth day, and the last degenerate cysts on the twenty-first day. More cysts were found than in any of the other animals of this group, but no symptoms were noted. On the twenty-first day it was re-inoculated and one normal cyst was seen on the tenth day, very few normal and degenerate cysts on the eleventh, none on the twelfth, very few normal and degenerate on the thirteenth, but on the fourteenth mucus was found, containing large numbers of normal and only a few degenerate cysts. Formed faeces on the same day contained only a few cysts. On the following two days only a few normal and degenerate cysts were seen, and from the seventeenth to twenty-first days only a few degenerate cysts. This is the only case in which mucus was found in any re-inoculated animal.

During the latter part of the work reported here several animals died which had not been recently infected. These guinea pigs had all received at least two inoculations of *E. caviae*.

An intercurrent bacterial infection was at first suspected, but most of the cultures made from the organs of the animals remained sterile. In one instance *B. paratyphosus* was isolated from the spleen. However, although repeated cultures were made from other animals using tetrathionate enrichment broth and eosin methylene blue plates in an endeavor to again isolate this organism, its presence was not again demonstrated. In cultures from three animals, *B. coli* was isolated from the spleen.

On autopsy the animals in this group showed one constant change. This consisted in the presence of severe hemorrhage in the mucosa of the small intestine, particularly in the region of the duodenum. The regional lymph nodes were usually enlarged and somewhat hemorrhagic.

Lesions which would indicate the presence of any of the commoner bacterial parasites of guinea pigs were not found in these animals. The relation of the previous coccidial infections to the death of these animals was likewise not determined.

A study of Andrew's protocols (1926) reveals the fact that seven out of the nine dogs and cats experimentally infected and subsequently re-inoculated with *Isospora felis* died at intervals of from five to sixty-one days after the last inoculation. No mention is made of the possible cause of death other than the statement that coccidiosis was not responsible. It is possible that some of these deaths may be explained on the basis of allergic reactions, which will be considered below, or they may be comparable to the death of the guinea pigs just described.

To summarize the results of attempts to reinfect recovered guinea pigs, it can be seen from a study of tables 5–9 that there are considerable variations in individual animals. The most noticeable differences from a primary infection in normal guinea pigs are the effect on the prepatent period and, with few exceptions, the entire lack of symptoms. In many cases the prepatent period is delayed from twelve, usually thirteen, to sixteen days; in a few cases the prepatent period is shortened, and in others it is normal, namely, eleven days. These results cannot be explained as resistance due to greater age of the animals, as some of the animals fed cysts for the first time were as old, and one (eight months old) was much older than any used in these experiments.

The results would probably have been more clear-cut if all of the animals used had been experimentally infected, so that the entire history were known in each case. However, this was impossible, as all the purchased animals had been previously infected and the animal colony was too small to provide sufficient animals for the experiment.

In the re-inoculated animals the occurrence of degenerate cysts at the beginning of the infection was very common, although in many cases no cysts had been found for many days before re-inoculation. For this reason it does not seem possible that the degenerate cysts found were the result of the previous inoculation. The more probable explanation of the occurrence of degenerate cysts at this time seems to be that whatever factors are concerned in the production of such forms toward the end of an infection are still active in the host at the time of re-inoculation. If this is the case, it is necessary to explain why normal cysts are produced later. It might be possible that the con-

tinued production of merozoites would be sufficient to overcome the immune(?) substance which causes the formation of degenerate cysts so that normal cysts are produced. However, this explanation would not suffice for all of the results, as in all cases there was a decided decrease in the number of oocysts produced in re-infections, unless a dual action was exhibited, namely, inhibition at some stage of the life cycle and production of degenerate cysts. Further work is required to elucidate these phenomena.

As mentioned previously, the intensity of the primary infection, the lapse of time between the first and second inoculation, and the period between the last passing of cysts and subsequent infection may all have some bearing on the occurrence of degenerate cysts and the other manifestations of immunity. However, the number of animals used does not permit generalizations on this phase of the problem.

A sufficient number of animals were re-inoculated while still passing cysts, however, to indicate that in these cases the prepatent period is shortened or complete immunity exists, at least, for the time being.

That resistance to subsequent inoculations is conferred by infection with *E. caviae* is quite evident. A total of thirty-five guinea pigs at various intervals after recovery were re-inoculated and, with the exception of one animal, failed to show clinical manifestations of coccidiosis equal to those observed in all initial infections. The course of the disease, as indicated by the occurrence of cysts in the faeces, similarly indicated that resistance or immunity was present in the re-inoculated animals.

As a rule, guinea pigs fed cysts three or more times showed an even lighter infection than those inoculated twice, if the number of normal cysts passed may be used as an index of severity of infection. The results of a number of re-inoculations will be discussed more fully below.

Allergic Manifestations

A preliminary report of the occurrence of allergic reactions following a subsequent inoculation of coccidial oocysts in guinea pigs which have recovered from coccidiosis has been made by Henry (1931c). A more detailed account of this process follows.

A study of tables 5–9 shows that in some instances guinea pigs re-inoculated with cysts died from two to ten days later. This occurred in nine cases in all. This data is compiled in table 10. In the first few deaths of this nature the guinea pigs were found dead in the morning.

But later several of them were watched prior to death. In all cases the clinical manifestations were similar. It may be of interest to give a resumé of the symptoms of one representative animal in detail. The day following re-inoculation, guinea pig E80 was quiet, appeared sick, and showed a loss of thirty-six grams in weight. The following day the weight was the same and the guinea pig was still sick. The third morning the animal remained in one position with its back arched. At 1:50 p.m. the animal began to sneeze and pawed at its nose with the front feet; the back legs were stretched out to the rear. The pawing lasted for about three minutes. Three such attacks occurred at ten-minute intervals. Between attacks the guinea pig moved around a little, squealed weakly, and continuously made movements as if eating, and wrinkled its nose. At 2:55 p.m. the guinea pig began bicycling, particularly with its front legs; more often the back legs were stretched out. Heavy breathing and choking sounds accompanied these movements. The head was thrown back. Sometimes the animal lay on its side, sometimes on its back. This occurred more or less continuously from 2:55 to 4:10 p.m., when the animal died without having gained its feet. The other animals that were observed while dying behaved in a similar manner, with the exception that loss of weight was noted in only two of the other eight animals.

At autopsy these guinea pigs did not present a very striking picture. The surface of the lungs usually appeared hyperemic and, on sectioning, the tubes were found to be distended with blood. In two cases the heart was somewhat enlarged. In every instance the liver, gall bladder, spleen, and kidneys were normal in appearance. The mesenteric lymph nodes were often enlarged and occasionally hemorrhagic. In some, numerous petechia were found on the mucous surface of the small intestine, and in all, petechia were found on the mucosa of the colon. The number of small hemorrhages varied greatly in the different animals. Usually the caecum presented no change. Infrequently, the stomach had a few hemorrhagic regions.

Cultures of the lung, liver, spleen, and heart blood made on a cooked blood agar medium remained sterile except in one case. This animal, found in the morning, had been dead several hours, and the Streptococci and *B. coli* which were obtained from the heart blood and spleen may safely be disregarded as agonal or *post mortem* invaders.

In four of the nine animals which exhibited anaphylactoid symptoms when coccidial oocysts were administered orally, degenerate cysts were present at the second inoculation. The initial infections of these

TABLE X

ANIMALS SHOWING ANAPHYLACTOID MANIFESTATIONS AS A RESULT OF RE INOCULATION WITH COCCIDIA

No. of guinea pig	No. of times previously inoculated	Last cysts	Days between last feeding and shock dose	Symptoms	Died	Remarks
E24	1	28 degen.	45	none	10	Few degenerate cysts found in contents of large intestine; slight loss of weight last two days.
E28	2	23 degen.	23	sick quiet	3	Fed second dose in morning; died in afternoon.
E36	1	27 degen.	27	none	2	Cysts in villi of caecum and large intestine; cysts in contents (those fed?)
E48	4		2 months	loss of appetite day died	2	No cysts found after last feeding.
E61	in lot of infected animals 2 months previously		about 2 months	none	2	Extensive petechia on large intestine.
E72	same lot as E61—also fed 1	21 degen.	21	heavy breathing and sneezing	9	No cysts seen.
E80	probably infected before purchase fed 1	21 degen.	27	quiet, sick second day	3	
E83	same lot as E80; fed 1	27 degen.	27	quiet, 1st day after feeding	8	Medium number of degenerate cysts found sixth day. Medium number of degenerate cysts in contents of caecum and colon.
E84	same lot as E80			none	9	

In each case the number indicates the days after feeding cysts.

animals had occurred twenty-one, twenty-three, and twenty-seven (two animals) days previous to the second inoculation. The other five animals were not passing cysts at the time of the second feeding, which occurred twenty-seven, forty-five, and approximately sixty days (two animals) after primary infection in the case of four of the animals. The data of previous infection was not known in one case.

In an attempt to produce this phenomenon by introducing the antigen by different routes three animals were inoculated subcutaneously with 0.75 cc of concentrated cysts. The antigen was prepared by using Sheather's sugar concentration method, by which, in addition to the concentration of oocysts, many of the bacteria and much of the debris (by careful washing) may be removed. However, the antigen still contained many bacteria. Two of the guinea pigs used (E76 and E87) (see table 8 for history) had been fed cysts one month previously and the last cysts (degenerate) were found in one animal ten days, and in the other twelve days, before the inoculation. The former animal had had a much heavier infection than the latter. The other guinea pig (E65) (see table 6 for history) had been fed cysts thirty-three days previously and eighteen days had elapsed since the last cysts were found. None of the three animals showed any sign of shock following the subcutaneous inoculation.

Next, an attempt was made to sensitize animals by subcutaneous inoculation. Two normal animals were inoculated subcutaneously with 1.5 cc of concentrated cysts. Twenty days later both were inoculated interperitoneally with concentrated oocysts (see technique under skin tests for method of preparing this antigen), one with 1.0 cc and one with 0.5 cc of the material. Within two minutes following the inoculation both animals showed anaphylactoid symptoms—sneezing, eating movements, violent movement of the front paws, rubbing their noses, blinking of eyes, etc. For the most part the animals remained on their feet, but at intervals one or the other would be prone for a few minutes. Both animals showed the above symptoms intermittently until one hour and a half after the inoculation. At this time both were lying on their sides and scarcely moving. They were frightened by a sudden noise near their cans and both resumed a sitting posture. The guinea pig that received the larger dose of antigen seemed weak the rest of the day and did not eat until the following morning. The other guinea pig occasionally made spasmodic movements with its legs during the afternoon. It also was weak and did not eat until the following morning. Three coccidia-free guinea pigs inoculated at the same time and

with the same antigen showed no symptoms other than a transient discomfort which lasted five to ten minutes.

The symptoms described above which occurred both in the animals which received a shock dose of coccidial cysts by mouth and by peritoneal inoculation so closely resemble those found in true delayed anaphylaxis that the phenomenon deserves considerable attention and further study.

The number of animals used in these preliminary trials has convinced the author that an allergic reaction occurs in coccidial infections. However, because of the crude manner in which the antigen was prepared, the possibility of bacteria playing some rôle in the manifestations described cannot be disregarded. In those animals in which the anaphylactoid symptoms were induced by the oral administration of oocysts it is difficult to understand how the bacteria contained in the antigen could produce these symptoms, while bacteria of similar kinds and in large numbers, always present in the intestinal tract, had not induced these symptoms previous to the time of second inoculation with cysts.

Preliminary experiments with the Dale-Schulz tests resulted in negative findings. Uterine and gut strips from guinea pigs inoculated subcutaneously and intraperitoneally with concentrated coccidial oocysts showed no reaction when this antigen was added to the bath. Likewise uterine and gut strips of normal animals did not react, which clearly proves that the antigen was not toxic. The failure to obtain a positive Dale-Schulz reaction with a whole antigen is in accord with the results of workers with bacterial antigens. An attempt is being made to purify and concentrate the coccidial antigen before further work with the Dale-Schulz technique is undertaken.

The stage in the life cycle of the protozoan parasite which is responsible for the initial sensitization and which acts as the shock antigen has not as yet been determined. It is logical to assume that the sporozoites which actually penetrate into the tissues of the host and whose proteins therefore necessarily produce cellular response should be the forms concerned. The induction of the allergic reaction by parenteral inoculation of cysts does not preclude the above assumption. In the preparation of the antigen used many of the mature cysts and the contained sporocysts were ruptured and therefore free sporozoites were available for absorption in the host.

Another possibility which should not be overlooked is that the oocyst may be involved. The permeability of the intestinal mucosa of

an animal recently infected with coccidiosis cannot be doubted after sections of such material have been examined. In many cases the epithelial lining of the mucosa is destroyed over large areas and the cells beneath riddled by the invading parasites.

So far as the author has been able to discover, no reactions similar to those described above have been reported for the protozoa. However, in preliminary experimental infections in swine symptoms similar, although less marked, have been observed in the re-inoculation of animals with two of the four species of coccidia occurring in this host. Considering the well known susceptibility of guinea pigs to anaphylactic shock, it is only reasonable to believe that like conditions occurring in another host might produce less characteristic and definite reactions.

SKIN TESTS

Active response on the part of the host when invaded by the coccidial parasite is further demonstrated by the results of skin tests performed on guinea pigs at various intervals subsequent to complete recovery (see table 11).

The antigen used in performing the skin test was concentrated and washed in a manner similar to that employed in the preparation of cysts for use in the subcutaneous and peritoneal inoculations previously described. In addition, the surface of the cysts, as well as the menstrum, were rendered sterile by the addition of full strength hexyl resorcinol for thirty minutes. The germicide was removed by thorough washing and the cysts again concentrated by centrifuging. As a result of the repeated centrifugization at high speeds, a large number of the cysts were ruptured. In performing the test 0.1 cc was inoculated intradermally on the abdomen of the guinea pigs in an area previously shaven.

Unfortunately, only one animal which was infected at the time of the test was included in the group tested intradermally. The reaction in this case was definite and terminated in the death of the animal within twenty-four hours after the injection. At the point of injection there appeared a blanched area 7 mm. in diameter, surrounded by a bright red zone 8 mm. in width. The entire area was approximately 2 mm. thicker than the surrounding skin. On autopsy, in addition to typical lesions of coccidiosis, the lungs were congested and on section showed blood in the tubules.

TABLE XI

SKIN REACTIONS OF NORMAL AND PREVIOUSLY INFECTED GUINEA PIGS

No.	No. of inoculations previous to skin test	Days since last inoculation	Days since last cysts	24 hours	48 hours
E62	1	60	39	red area 4x4x2	red area 6x7x2 sl. white halo
E65	1	52	37	sl. thickened	7x6x3 pink
E66	1	52	37	yellow area 4x4x3	yellow area 4x4x3
E67	1	52	36	yellow area 4x4x3	yellow area 4x4x3
E76	1	52	8	sl. yellow zone	yellow zone 9x9
E77	1	52	24	sl. thickened	5 mm. yellow zone
E78	1	52	28	sl. yellow zone	sl. reddened 10 mm. dia.
E86	1	49	28	3x3x1 pink	10x8x2 pink
E87	1	49	28	....	4x3 sl. red
E88	1	49	12	12x12x2 white	12x12x2 white
E91	1	32	passing cysts	12x15x2 very red— central blanched area—died	
E95	1	22	2	2 mm. blanched area, sl. thick.	6x7, red sl. thick.
E60	2	23	4	.. ..	7x10, thickened pink halo
E64	2	19	3	4 mm. red area, thickened	10x6x1 red with yellow halo
E68	2	21	4	6x8x1 red	thickened-red with diffuse yellow zone
E69	2	21	4	6x5x1 red	6x5x1 red
E70	2	32	10	8x10x3	10x15x1 diffuse red
E75	2	32	9	sl. yellow zone	1 mm. raised area surrounded by 10 mm. yellow zone
E82	2	18	3	4x4x2 blanched	7x7x2 red halo around yellow zone
E89	2	18	passing very few degenerate cysts	....	thickened
E59	3	19	3	4 mm. thickened area	5x8x1 red center yellow halo
E100	....	....	. ..	3x3x2 pink	6x6x1 sl. red
E102	. ..	... .	. .	1 mm. pink	5x5x1 pink
E104	....	... .	....	2x2x2 pink	killed
E106	....	. .	. ..	4x4x2 pink	6x6 sl. pink

While there was a considerable non-specific reaction resulting from the injection of this antigen, as indicated by the swellings produced in the normal animals, sufficient difference was noted between the reactions in the normal animals and those induced in the previously infected animals to suggest the presence of an immune reaction (see table 11).

In general, those animals which have most recently ceased passing oocysts showed the greatest change. The guinea pigs which had not passed cysts for a period of four weeks or more previous to the intradermal injection showed little or no greater reaction than did the normal animals. The interval between first inoculation and intracutaneous injection and the number of preceding infections apparently had no bearing upon the response to the skin test, except in so far as they bore a relation to the interval of passing of cysts and testing.

The most interesting result of these preliminary tests would seem to be the confirmation of the presence of an allergic phenomenon connected with coccidial infection, indicated by the results on the single animal actually infected at the time of the test.

Re-Inoculation After Intradermal, Subcutaneous, and Interperitoneal Injection of Coccidia

One week after the intradermal inoculations were made as reported in the preceding section thirteen of the animals, together with six others, were fed oocysts. This latter group consisted of five normal guinea pigs and one (E92) which had been inoculated subcutaneously with a concentrated suspension of oocysts 28 days previous to feeding and also had been injected intraperitoneally with a similar suspension of cysts 7 days previous to feeding. At the time of the intraperitoneal inoculation allergic symptoms not discernible in the untreated controls were manifested by this animal. The previous histories of these animals as well as the results obtained by the last inoculation are shown in table 12.

It is evident from the results in table 12 that the two animals (E103 and E106) not infected but which received intradermal injections showed no effect of this injection in so far as the prepatent period was concerned. Normal cysts only were passed in their faeces and the clinical symptoms of coccidiosis were identical with those of animals receiving initial infection.

That the delayed prepatent period shown by re-inoculated animals was not influenced by an intervening inoculation by the intra-

TABLE XII

RESULTS OF ORAL INOCULATION OF GUINEA PIGS WITH COCCIDIA, ONE WEEK AFTER GIVING SKIN TEST

No.	No. of times previously fed	Days after last feeding	Days since last cysts passed	First cysts	Type of cyst first passed	Symptoms	Remarks
E60	2*	30	12	12	1 degen.		No cysts 15th day.
E62	1*	67	48	13	degen. and normal		15th day few cysts; almost all degenerate.
E66	1*	61	50	12	few, degen. and normal		15th day very few; all degenerate.
E67	1*	61	53	12	very few normal		15th day few cysts; almost all degenerate.
E76	1	61	17	12	normal		15th day, few cysts; few degenerate.
E77	1	61	32	11½	normal	diarrhoea 12th–13th	Fed 1 large dose. 15th day few cysts, mostly degenerate.
E78	1	61	30	13	few normal		Fed 300,000 cysts. 15th day very few, normal and degenerate
E82	2*	26	9	....	....		Up to 15th day no cysts.
E86	1*	57	36	12	normal		Coccidial antigen injected subcutaneously 26 days before second feeding.

* First infection was natural.
Number in each case indicates days after feeding.

TABLE XII—*Concluded*

RESULTS OF ORAL INOCULATION OF GUINEA PIGS WITH COCCIDIA, ONE WEEK AFTER GIVING SKIN TEST

No.	No. of times previously fed	Days after last feeding	Days since last cysts passed	First cysts	Type of cyst first passed	Symptoms	Remarks
E89	2*	26	6	....	....		Up to 15th day no cysts.
E95	1	30	6	12	1 normal		15th day very few, mostly degenerate.
E92	....	....	....	13	1 normal		Injected subcutaneously 28 days previously, intraperitoneally 7 days previously. 1 normal 13th day only cyst seen.
E103	....	....	....	11½	normal	diarrhoea	Skin tested.
E106	....	....	....	11½	normal	diarrhoea	Skin tested.
E98	....	....	....	11½	normal	diarrhoea	Not skin tested.
E101	....	....	....	11½	normal	diarrhoea	Not skin tested.
E108	....	....	....	11½	normal	diarrhoea	Not skin tested. Died 15th day.
E113	....	....	....	11½	normal	diarrhoea	Not skin tested.
E114	....	....	....	11½	normal	diarrhoea	Not skin tested. Died 13th day.

* First infection was natural.
Number in each case indicates days after feeding.

cutaneous method is also clearly seen by a study of the eleven animals treated in this manner. In general, the results duplicate those shown in tables 5 to 9.

Of particular interest in this group are the results of the oral inoculation of guinea pig E92. Although this animal had never received cysts per os, it showed a greater resistance than did most of the guinea pigs infected in this manner. The prepatent period was thirteen days and at this time a single cyst was detected although careful search was made. Aside from this one oocyst, there was no indication whatsoever of infection in this animal. Since the five animals used as controls in this series, as well as the two which had received only a skin test, developed typical cases of coccidiosis, it seems more than a coincidence that E92 was practically immune. The possibility of producing immunity through parental inoculation of oocysts is being further investigated. Heretofore, this method has failed to produce immunity. Among others, Tyzzer (1929) was unable to immunize chickens against *E. tenella* infection by intravenous injection of large numbers of merozoites.

EPIDEMIOLOGY

It is interesting, in the light of the results obtained by experimental infections, to return to the epidemic of coccidiosis in the colony of guinea pigs which first attracted attention to this problem.

Coccidiosis was discovered in a colony of approximately three hundred animals at the Division of Veterinary Science, University of California. Through the courtesy of Dr. C. M. Haring of this Division, the author was privileged to examine the infected animals.

Most of the guinea pigs in this group had been inoculated with milk or other bovine materials and were kept in individual containers for practically their whole stay in the colony. The cages were in three tiers which were arranged in double rows with approximately four feet separating each of these units.

In all, twenty-one animals died during the original and two subsequent outbreaks. When the first indications of coccidiosis were recognized, a direct examination of the faeces of sixty-six guinea pigs which were in close proximity to the fatal cases showed that thirty-two of those examined had cysts of *E. caviae* in their faeces. By the large number of cysts in those guinea pigs which had been kept close to the fatal cases as well as by the fact that some deaths in this group followed, it was seen that a focus of infection had been established, with

the position of the animals which had first succumbed as a center. The spread of the infection was logical, in that new infections appeared on both sides and particularly below the original focus.

In the course of the experiment for which these animals had been inoculated this group was killed and the epidemic of coccidiosis did not spread to the other animals in the colony to any extent.

Recurrences of the epidemic occurred, on a smaller scale, from time to time whenever conditions in the colony became such that sufficient drying of the containers was not accomplished. As a matter of routine the cages in which the animals were kept were changed at four-day intervals, washed, scrubbed with a disinfectant, and allowed to drain and dry for four days. When the colony was unusually large, this period of drying was of necessity considerably shortened. It was at these times that coccidiosis became evident. Successful control measures consisted merely in prolonging this drying period.

In a colony in which the animals are being rapidly acquired and disposed of the long prepatent period of *E. caviae* and the comparatively short time during which normal cysts are passed both serve to prevent a rapid spread of the disease. It is obvious that the first of these two factors does not function in a relatively permanent colony.

MULTIPLICITY OF SPECIES

Throughout this report warning has been repeatedly given concerning the difficulties which arise from the presence of more than one species of coccidium in a given host. This possibility in the case of coccidiosis in guinea pigs has received considerable attention and there are some indications that during the latter part of the work two species may have been involved.

In addition to the large form described as *E. caviae* by Sheather (1924), a smaller form has been found in a few animals of one group (pl. 11, figs. 4–6). Associated with the presence of this atypical type were lesions in the host somewhat different from those encountered in animals in which only the larger form was recognized. However, none of these animals in which the smaller cysts were present and which showed the lesions mentioned were entirely free of the typical form of *E. caviae*.

Pathologically the two types seem to be differentiated by the occurrence of excessive oedema and gelatinous infiltration in the mesentery surrounding the first loop of the colon, with considerable quantities of

mucus in the lumen of the intestine in this region, when the smaller form is found to predominate. The mucoid masses in these cases consist for the most part of packed small cysts.

Although it will not be possible to determine definitely the prepatent period of the smaller type until it can be completely separated from the larger, it has been noted in three instances that the cysts of this form appear in the faeces approximately two days later than the larger type.

CONCLUSIONS

A study of the life cycle of *E. caviae* has been made, which confirms the major portion of the work reported by Sheather. In addition to invasion of the colon, which Sheather has reported, stages of *E. caviae* have been found in the caecum and small intestine of infected guinea pigs.

It has been shown that the sporulation time of a coccidial species is of considerable value as a distinguishing characteristic when proper precautions are taken to sufficiently standardize conditions. Under proper environmental conditions, sporulation time for *E. caviae* is two to three days. This is not in accordance with the findings of Sheather, but no comparison may be made because of the difference in methods used.

The prepatent period has been found to be exceedingly constant in previously uninfected animals, with the usual variation of only a few hours in the eleven and one-half day period. That great variations in this period are due to resistance in the host as a result of previous infection, or to contamination of the faeces of the animal examined, seems exceedingly probable. It has been clearly shown that the former factor may be expected to influence the prepatent period in almost all cases. That contamination by oocysts may occur is easily understood by workers who have had experience with experimental infections with any of the coccidia.

The definite symptoms, the acute nature of some cases, and the widespread occurrence of coccidial infections in guinea pigs make this disease one of considerable interest and importance to laboratory workers and to a large number of persons engaged in the raising and marketing of these animals. Although the mortality is relatively low as compared with several other coccidial infections, the aftermath of even a light infection is such as to cause a large economic loss.

Although the pathogenicity of *E. caviae* has been questioned, it is clear from the work presented that great virulence is exhibited by this

organism in some cases. The clinical symptoms, such as diarrhoea and emaciation, leave no doubt concerning the ability of this parasite to injure the host. This is further borne out by the lesions encountered in the intestinal tract, particularly in the colon. Petechial hemorrhages on the mucous surface of the upper end of the colon are the most constant lesions found.

It is only upon microscopic examination, however, that the real extent of the damage is discovered. Entire areas of the mucosa may be denuded of their epithelial lining, or the infection may be so heavy that every cell contains some stage of the parasite.

The mortality in infections in which all other agents have been eliminated as possible factors amounts to as high as 40 per cent.

Definite indications have been obtained that a true resistance is developed in the body of the host by the establishment of an infection. The re-inoculation of sufficient numbers of previously infected animals has been performed to warrant definite conclusions. While the immunity developed has varied in degree, in no case has there been a failure to show that some protection has been provided by the previous infection. In a few cases a solid immunity was developed.

An expression of immunity which has not been described before in coccidial infections is presented in the case of the formation of degenerate cysts, which occurs toward the end of an initial infection, or in the earlier as well as in the later stages during second invasion by the parasite.

Although the possibility of bacterial proteins entering into the reaction has not been completely eliminated, it seems highly probable than an allergic reaction has for the first time been demonstrated in the protozoa. This reaction has been shown to occur when sensitization has been attained by the oral and parental inoculation of the proteins concerned. The shock dose has been effective when introduced into the digestive tract or the peritoneal cavity, as well as when inoculated intracutaneously.

Except for the fact that allergy was definitely demonstrated in one case, skin tests have not been sufficiently developed to provide a basis for conclusions concerning immunity. It is clear, however, that animals which have recovered from an infection with *E. caviae* relatively recently exhibit cutaneous reactions which are dissimilar to those shown by uninfected animals and by animals which have not passed cysts for a considerable length of time.

It seems possible from the evidence presented that two species are concerned in coccidiosis in guinea pigs.

SUMMARY

1. A study has been made of *E. caviae* infection in guinea pigs.

2. The work of Sheather concerning the life cycle of the parasite has been confirmed.

3. Sporulation time of the oocysts of *E. caviae* has been found to be two to three days.

4. The prepatent period has been shown to be definite and does not vary more than a few hours from a period of eleven and one-half days in initial infections.

5. The course of the infection and the clinical symptoms of the disease produced in guinea pigs by *E. caviae* have been studied. Diarrhoea and emaciation have been found to be the most constant symptoms.

6. A study of the gross and microscopic pathology has been made. Intestinal hemorrhages, sloughing of mucous lining, and enlargement of regional lymph nodes are the outstanding macroscopic characteristics. Invasion and development within the cells of the mucosa has been demonstrated by microscopic examination.

7. Immunity has been demonstrated by the re-inoculation of thirty-five previously infected animals.

8. Hypersensitivity to coccidial proteins has been shown to exist in animals previously infected and has been produced by parental injection.

9. A demonstration of this hypersensitivity has been possible by oral, intraperitoneal, and intracutaneous inoculation of shock dose.

10. Skin tests have been performed upon a group of infected and recovered animals, with the result that definite though slight reactions were found in those animals which had recently recovered.

11. A study of the morphology and physiology of the coccidia of guinea pigs has indicated the possibility of the occurrence of more than one species in this host.

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EXPLANATION OF PLATES

PHOTOMICROGRAPHS BY J. E. GULLBERG

Magnification $\times$ 1600

PLATE 11
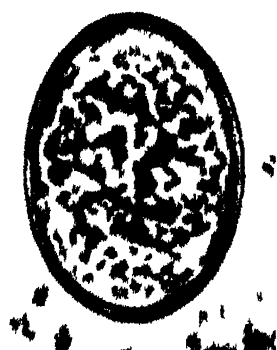
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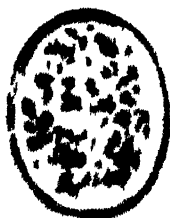
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PLATE 12

Magnification × 1600

Fig. 1. Degenerate oocyst containing large globules, particularly on one side.
Fig. 2. An end view of merozoite cyst in a section of the colon.
Fig. 3. Degenerate oocyst with globules filling entire cyst wall.
Fig. 4. Two degenerate oocysts, one showing rift in cyst wall.
Fig. 5. Section of colon showing two merozoite cysts.
Fig. 6. Two degenerate oocysts; the upper shows globules in center of oocyst; the lower oocyst contains homogeneous material completely filling oocyst wall.
Figs. 7 and 8. Sections of colon.
Fig. 7. A microgametocyte enclosed in an epithelial cell. The nucleus of the host cell is pushed to one edge of the cell.
Fig. 8. A macrogametocyte in invaded cell. The nucleus of the macrogametocyte is in the center.
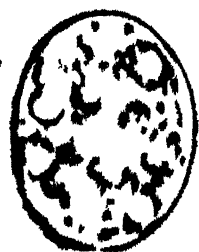
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7 8

PLATE 13

Figs 1–3   Sections of the large intestine
   Fig 1   Low power of a section showing heavy invasion of *E caviae*
× 275
   Fig 2   Higher power of region near muscularis (seen in lower left hand
portion)   Practically every epithelial cell has been invaded by the parasite
× 560
   Fig 3   Higher power of one region near the edge of the mucosa   Hemor
rhage just beneath the surface can be seen   × 560

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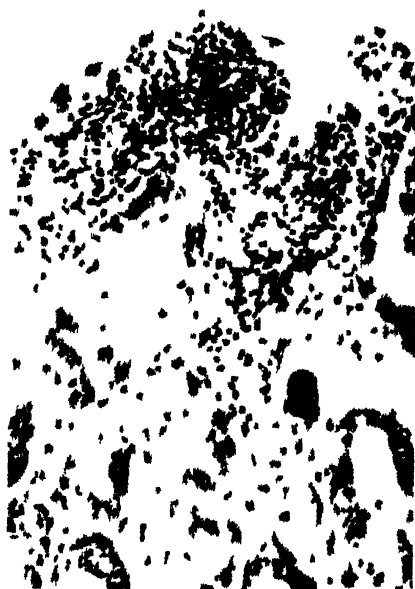
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PLATE 14

Figs. 1–4. Sections of the large intestine.
   Figs. 1–2. Sections of heavily parasitized glands. Most of the epithelial cells have been sloughed off. Interior of gland plugged with oocysts. × 1100.
   Figs. 3–4. Sections through white plaques.
      Fig. 3. Accumulated oocysts surrounded by lymphocytes. × 325.
      Fig. 4. Higher magnification of same region. The oocyst walls are crumpled due to ineffective fixation. × 1100.

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THE OOCYST WALL IN THE GENUS EIMERIA

BY

DORA PRIAULX HENRY

THE OOCYST WALL IN THE GENUS EIMERIA

BY

DORA PRIAULX HENRY

The appearance of the wall of the coccidial oocyst is one of the many criteria used in the differentiation of species. In a given species, as a general rule, the following characteristics of the oocyst wall are fairly constant; thickness, uniformity, texture (smooth or rough), color, and presence of accretions.

The wall of the oocyst is relatively thin in *E. mitis*, *E. debliecki*, *E. faurei*, and *E. zürnii*, while in others, as *E. maxima*, *E. intricata*, *E. bukidnonensis* (see Tubangui, 1931), and *E. scabra*, it is comparatively thick.

Uniformity in thickness of the oocyst wall is shown by *E. zürnii*, *E. cylindrica* (see Wilson, 1931), *E. debliecki*, *E. mitis*, and *E. acervulina*, whereas in *E. smithi*, *E. bukidnonensis*, *E. scabra*, *E. faurei*, *E. intricata*, *E. stiedae*, and *E. maxima* the thickness varies, the wall being much thinner at the micropyle end. Also, in some species the variation in thickness of the wall is much greater than in others; for example, in *E. maxima*, *E. tenella*, and *E. faurei* there is a slight thinning at one end, while in *E. smithi*, *E. bukidnonensis*, and *E. intricata* the difference in thickness is very great. In *E. magna*, according to Kessel and Jankiewicz (1931) the wall is definitely thicker at the micropyle end and thins out at the opposite end. With the exception of oocysts of *E. magna*, this characteristic is apparently correlated directly with the micropyle. Where no micropyle or a very inconspicuous micropyle is present, very little or no thinning of the oocyst wall is found. In most cases, however, in which a micropyle is present, the oocyst wall is somewhat heavier at the pole opposite this opening.

Another obvious and readily recognized specific characteristic is the texture of the oocyst wall, i.e., whether it is rough or smooth. Some forms have entirely smooth walls, as *E. mitis*, *E. acervulina*, *E. zürnii*, and *E. faurei*, while others, such as *E. scabra*, *E. perminuta*, *E. intricata*, *E. maxima*, *E. bukidnonensis*, *E. canis*, *E. clupearum*,

and *E. sardinae* (see Wenyon, 1926, for descriptions of the last three species) show a considerable degree of roughness.

The walls of the oocysts of the last mentioned species vary considerably in the degree of roughness, with *E. maxima*, the wall of which is only slightly irregular, at one extreme and *E. intricata* or *E. bukidnonensis* at the other. At least one species (*E. spinosa*, Henry, 1931) has been described in which the irregularity of the wall is so marked that the outer surface is covered with spine-like projections.

Another species-differentiating characteristic is the color of the oocyst wall. In many species the oocyst wall is colorless, as in *E. zürnii, E. ellipsoidalis* (see Becker and Frye, 1929), *E. mitis, E. acervulina,* and *E. perforans.* Colors ranging from yellow to brown are most commonly found. The oocyst wall in *E. faurei* and *E. sardinae* is yellowish, in *E. maxima* and *E. clupearum* it is brownish, while in *E. intricata, E. scabra,* and *E. spinosa* it is quite brown. The wall of *E. tenella* appears greenish, while that of *E. smithi* is salmon pink. Also, Bruce (1919) has described a species in rabbits, which has a pinkish orange color. Wenyon (1923) described *E. canis,* which has a red or pink wall. It is rather interesting that in most of the forms which have colored oocyst walls, the walls are rough.

In some species the oocyst wall has a tendency to collect extraneous material from the surrounding medium. This is true of *E. maxima, E. sardinae,* the form described by Bruce (1919) in rabbits, and to some extent, *E. caviae.*

The multiple cyst wall which is characteristic in the oocysts of the genus *Eimeria* seems to the author to be exceedingly interesting and of considerable significance, yet it has been neglected by the majority of workers who have studied this genus. According to Labbé (1896), Eimer described a wall for *Eimeria falciformis* which consisted of an outer resistant capsule and an inner thin envelope surrounding the protoplasmic body. Labbé considers this a characteristic of the genus *Eimeria.*

In textbooks and many papers on the oocyst of the genus *Eimeria* the oocyst is described simply as having a double-contoured cyst wall. Yet in many species careful study of the wall of the oocyst has shown it to consist of two quite different membranes. In a few cases three membranes have been seen.

The purpose of this paper, therefore, is to present the results of examining in detail the tri-part oocyst wall of one species, to determine to some extent the distribution of this phenomenon in various

species of *Eimeria,* and to show the separate membranes by means of photomicrographs.

For the careful and detailed study of this structure the oocysts of *Eimeria intricata* were used, chiefly because they are relatively very large (35.2–51.2μ in length and 28.8–35.2μ in width). They are very brown in color and the oocyst appears very rough and irregular (pl. 15, figs. 1 and 2). There is a large micropyle and above this a large, clear, and glassy polar cap is usually seen. This description agrees with that of Spiegl (1925) and of Sheather (1926).

The outer surface of the oocyst consists of a transparent membrane which is colorless. As Sheather has already pointed out, it is uneven in thickness, often on a single oocyst. In most oocysts it was difficult to determine whether this was a continuous membrane or merely accretions. Occasionally oocysts in which the internal part had shrunk were found as shown in plate 15, figure 3. In this figure the outer membrane, which is very thin, is clearly seen. In the region of the micropyle the polar cap can be seen as a thickening of this membrane. Lerche (1921) did not believe that the polar cap in *E. faurei* was a thickening of the mucous covering of the oocyst. This outer covering is very fragile and easily slips from the oocyst. For this reason, *E. intricata* oocysts upon which a polar cap cannot be detected are fairly common. This layer, excluding the polar cap, is very thin (0.2–0.4 μ) and has the appearance of a mucoid material. The lack of rigidity shown by the irregularities described above further strengthens the assumption that this wall is mucoid in nature.

Sheather's description of this outer membrane differs from the one here given in that he describes the outer membrane as pale yellow in color, with a rough surface, and gives its thickness as varying from 1 to 2 μ in the same oocyst. It is possible that this variation in thickness was owing to the fact that measurements were made from the outer surface of the first membrane to the outer surface of the second wall. As shown in plate 15, figure 3, this distance is not always constant, because of the separation of the two layers at various places on the oocyst.

Spiegl recognized two walls, the outer of which had a delicate wrinkling. Which of the two outer membranes he referred to cannot be determined.

Inside the transparent outer membrane (pl. 15, figs. 3 and 4) is a thick, rough brown layer of from 2.0 to 2.5 μ in thickness. A high focus (pl. 16, fig. 9) shows the irregularities of this wall, as the outer

membrane is transparent. The intermediate layer thins out at the smaller end of the oocyst, where a micropyle of from 7.5 to 9.5 μ in diameter is found (pl. 15, fig. 1). Sheather states that this layer is constantly 1 μ thick and believes it is rough, but considers it possible that it may be the rough outer layer. It has been possible by exerting pressure to push the outer layer from the oocyst, in which case there is no doubt that it is the intermediate layer that is rough.

By exerting pressure on a cover-slip over a smear of *E. intricata* a third cyst wall became visible (pl. 15, fig. 4; pl. 16, fig. 7). This was most successfully performed, using a needle, when the cover-slip was rimmed with melted paraffin. Oocysts which had been kept in 2 per cent $K_2Cr_2O_7$ a few days and then centrifuged were the best specimens to use. Completely sporulated oocysts almost invariably broke and released the sporocysts, and sometimes even the sporozoites were released. In plate 16, figure 7, an oocyst is seen in which the intermediate layer which is rough and brown has been removed and is at the left. The oocyst consists only of the innermost layer.

This wall is relatively thin (0.8–1.0μ), colorless, and smooth. Inside this the four sporoblasts are seen. In another oocyst (pl. 15, fig. 4) mature sporocysts can be seen inside the innermost layer. In this case an even pressure was exerted on the oocyst and when the contents surrounded by the innermost layer had slipped halfway out of the crack in the intermediate layer, the pressure was suddenly released; the intermediate layer snapped back and caught the innermost layer, partly caving in the sides. After the photomicrograph was taken, the oocyst was forced entirely free of the intermediate layer, but the innermost layer was permanently collapsed. This represents the inner lining which Sheather suspected.

Eimeria faurei was examined in a similar manner, but as the oocysts are much smaller, greater difficulty was experienced in separating the layers. The oocyst is yellow in color, has a smooth wall, and a colorless polar cap (pl. 16, fig. 5). The polar cap is apparently a thickening of a thin membrane which surrounds the oocyst as in *E. intricata*, but I have been unable to separate it. The next layer contains the yellow color. When pressure is exerted this breaks open and the innermost layer is seen (pl. 16, fig. 6). This is thin and colorless, identical in appearance with that of *E. intricata*.

Yakimoff *et al.* (1927) described a new species, *Eimeria nina kohl-yakimov*, in sheep and in 1930 Yakimov and Rastegaieff reported it from goats. The oocysts of this species agree in size, shape, and color

with the oocyst of *E. faurei,* from which the two outer layers have
been removed. However, Yakimoff has found this new form when no
E. faurei was present, and the time necessary for sporulation differs
from that of *E. faurei.*

Previous to the study of the oocyst wall in the sheep coccidia, the
appearance of the large polar cap in both these species, but in none
of the other common species seemed to have some evolutionary sig-
nificance. This has been enhanced by the parallelism in the structure
of the oocyst wall in these two forms.

Following the study of the oocyst wall in the sheep coccidia, the
oocysts of all available coccidia were examined. The oocysts of *Eime-
ria bilamellata* (Henry, 1932a, pl. 18, fig. 12) have a wall with an
outer rough brown layer and an inner smooth white layer. *Eimeria
scabra* has a two-layered wall, which is very similar to that of *E bila-
mellata.* *Eimeria smithi* has an outer colored wall and an inner
colorless one. The oocysts of *Eimeria residua* (Henry, 1932a) also has
a brown, rough outer layer and a colorless smooth inner layer. The
wall of the oocyst of *E. caviae* is probably made up of two layers. In
fresh oocysts a double wall could not be seen, so oocysts were centri-
fuged at a high speed for fifteen minutes. An outside wall could not
be separated from an inner one; but fragments of the outer wall,
which had broken into many pieces as a result of the pressure, could
be seen.

In the oocysts of *E. maxima* a double-layered wall was not seen.
The wall has tremendous expansive powers.

It seems certain from the above examples that species whose
oocysts have complex walls are more common in the genus *Eimeria*
than has been thought heretofore. No doubt the structure of the cyst
wall of other species will be found to be similar.

That this multiplicity of walls is not confined to the genus *Eimeria*
is shown by the report of Wenyon (1926) of a double wall in *Isospora
belli* and by the results of the examination of oocysts of *Isopora lacazii*
(Henry, 1932b), which were found to have three layers.

The occurrence of separable walls surrounding the oocysts of coc-
cidia offers another means of identification in addition to those
commonly used.

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EXPLANATION OF PLATES

PLATE 15

Figs. 1–4. *Eimeria intricata* Spiegl from the sheep

Fig. 1. Non-sporulated oocyst, showing colorless polar cap. The cytoplasm cannot be seen because of the thick wall.

Fig. 2. High focus of same oocyst to show rough wall.

Fig. 3. Non-sporulated oocyst, with cytoplasm rounded into a ball. The outer, thin, colorless membrane can be seen surrounding the oocyst. Notice the thickening, representing the polar cap, at one end.

Fig. 4. Sporulated oocyst upon which pressure was exerted and released suddenly as the oocyst was half way out of the intermediate layer of the wall. The outer layer cannot be seen.
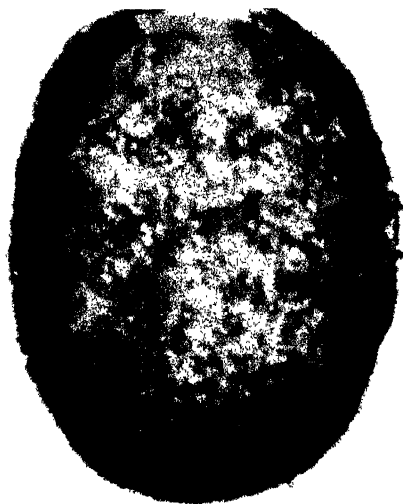
1

2

3

4

PLATE 16

Figs. 5–6. *Eimeria faurei* (Moussu and Marotel) from the sheep

. Fig. 5. Non-sporulated oocyst. The colorless polar cap is seen at one end.

Fig. 6. Oocyst upon which pressure has been exerted. The innermost layer can be seen surrounding four sporoblasts. At the right and partly underneath is the intermediate layer.

Fig. 7. Oocyst of *E. intricata* upon which pressure has been exerted. The thin, transparent innermost layer surrounds the four sporoblasts. The rough, intermediate layer lies to the left.
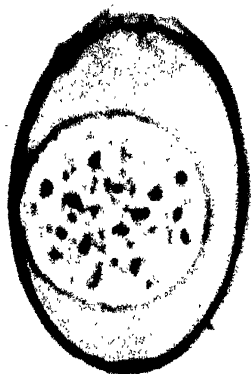
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6

OBSERVATIONS ON COCCIDIA OF SMALL MAMMALS IN CALIFORNIA, WITH DESCRIPTIONS OF SEVEN NEW SPECIES

BY

DORA PRIAULX HENRY

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The ubiquity of coccidia is well known as they have been found in almost every animal in which a systematic search for them has been made. With the exception of coccidia in domesticated animals, in most cases the oocyst alone has been studied. The coccidia of each mammalian species examined are considered as belonging to a separate species and the oocysts show certain characteristics that differentiate them from those of the coccidia of closely related species. Also, abounding evidence of a strict host-parasite specificity has been brought forth by failures in attempted experimental transfers. In fact, the only recognized instances in which the same species is found in different hosts is in the cases of the coccidia of the cat and dog and, probably, of sheep and goats. Yet in the few cases, with the exception of domesticated animals, in which animals of the same species have been examined from distant regions the coccidia have been found to be the same, i.e., coccidia in gray squirrels from England, Switzerland and California. Recently many workers who have studied the coccidia of animals have found more than one species in the animal studied. Tyzzer (1929) found four species in chickens, Henry (1931), four species in swine, Kessel and Jankiewicz (1931), five species in rabbits, and various workers have reported at least five species in cattle.

In the following pages are presented the data accumulated from the examination of a miscellaneous group of small mammals collected from various regions in California. The number of species found is in marked contrast to the single species of coccidia found in most of the birds encountered in the same geographical area (Henry, 1932b).

The time required for complete sporulation of the oocysts of the different species described is included in each description. It is neces-

sary, however, to point out that the time given is not necessarily the minimal time required for sporulation, as sporulation was not carried out under optimum conditions. Intestinal contents containing the oocysts were placed in 2 per cent $K_2Cr_2O_7$ in small vials in most cases. This method is not so efficient as placing the material in Petri dishes.

I wish to acknowledge the continued interest of Dr. C. A. Kofoid in this work.

EIMERIA OF GROUND SQUIRRELS

A new species of coccidium, *Eimeria citelli,* from the striped ground squirrel (*Citellus tridecemlineatus*) has been described by Kartchner and Becker (1930). Seventy-eight ground squirrels were examined and 20.5 per cent were infected with this coccidium. The oocysts were ellipsoidal, ovoid, or subspherical, the range in length was from 15 to 23 μ, and in width from 14 to 19 μ; the average size was 18.8 $\mu \times$ 15.8 μ.. The oocyst wall consisted of three layers, a thin ectomembrane, a thin endomembrane, and a thicker intermediate layer. In freshly passed oocysts the area between the protoplasm and the wall was distinctly pink. No micropyle was seen. Complete sporulation occurred in seventy-two hours. At this time a large residual body is present; in three or four days this shrinks in size. Experimental infections were performed.

Six ground squirrels have been examined; three of these belong to the genus *Citellus* and three to the genus *Callospermophilus.* Two species of *Citellus* were examined. One of these, *Citellus beldingi,* was negative for coccidia.

Eimeria beecheyi sp. nov.

The oocysts of *Eimeria beecheyi* sp. nov. (pl. 18, fig. 6) have been found in two ground squirrels (*Citellus beecheyi*) from Lake County and the vicinity of Nevada City, California. These ground squirrels were probably different varieties. In both animals the number of oocysts found was comparatively small, and in both the oocysts were found only in the caecum and large intestine. The oocysts varied in length from 16.0 to 22.4 μ and in width from 12.8 to 10.2 μ; the average was 19.2 $\mu \times 16.0 \mu$. The oocyst is ovoidal in shape; usually one end is slightly narrower than the other. The oocyst wall is colorless than about one μ in thickness. No micropyle has been seen.

The oocysts were completely sporulated in from four to five days. Rather large polar granules were found inside the cyst wall after formation of the sporoblasts, but no residual body. The absence of a residual body, and the color and shape of the oocysts differentiate this species from that described by Kartchner and Becker (1930).

EIMERIA OF THE GOLDEN MANTLED GROUND SQUIRREL

Three ground squirrels (*Callospermophilus chrysodeirus*) from Placer County, California, were found to be infected with coccidia. One was heavily infected with one species of coccidia and one heavily infected with another species, and the last ground squirrel had a very light infection of the latter species. These species will be described below.

Eimeria callospermophili sp. nov.

Oocysts of *Eimeria callospermophili* sp. nov. (pl. 17, figs. 3, 4) were found in large numbers in the contents of the intestines, particularly of the caecum. The oocysts ranged in length from 16.0 to 22.4 μ and in width from 16.0 to 22.4 μ; the average size was 19.2 $\mu \times 16.0$ μ. They are subspherical in shape and the cyst wall is slightly rough and yellowish in color. The wall probably consists of two layers, an outer, slightly rough, and an inner, smooth layer. All attempts to separate them have completely failed. Sporulation began on the sixth day after the oocysts had been placed in $K_2Cr_2O_7$ and was completed in most of the oocysts by the seventh day. When the oocysts were observed in the intestinal contents, the protoplasm entirely filled the cyst wall. After two days in the $K_2Cr_2O_7$ in about one-third of the oocysts the protoplasm had rounded up in the center of the oocyst. At this time a few granules were usually seen along the edge of the cyst. After the formation of the sporoblasts a residual body measuring from 3 to 5 μ is found. This is either homogeneous and clear, or granular. The sporocysts are almost round and pointed at one end; they measure about 10.2 $\mu \times 8.5$ μ. The residual material in the sporocyst is meager, consisting only of a few granules.

This form is more similar to *Eimeria citelli* described by Kartchner and Becker (1930) than is *E. beecheyi*. However, the oocysts of *E. callospermophili* have a two-layer wall which is rough and yellowish in color.

Eimeria bilamellata sp. nov.

Oocysts of *Eimeria bilamellata* sp. nov. (pl. 17, figs. 1, 2, 5) were found in two ground squirrels. These oocysts, which are egg-shaped, varied in length from 25.6 to 35.6 μ and in width from 22.4 to 25.6 μ; the average size was 32.0 $\mu \times 25.6 \mu$. The oocyst is enclosed in two walls (see Henry, 1932a); the outermost is brown, thick, and rough; the innermost is transparent, thinner, and smooth (pl. 17, fig. 5). A micropyle is present and the walls are thinner at this end of the cyst. The exact time required for sporulation was not ascertained for this species, as the material collected from the heavily infected animal did not show any sporulated cysts; fully sporulated oocysts, however, were found in the intestinal contents of the slightly infected ground squirrel ten days after the material was mixed with $K_2Cr_2O_7$, so that the time required for complete sporulation in this species is less than ten days. The sporocysts measure 16.0 $\mu \times 9.6 \mu$ and contain large amounts of residual material (pl. 17, fig. 2), but no residual body is present in the oocyst.

EIMERIA OF THE JACK RABBIT

Oocysts (pl. 18, fig. 7) were found in one jack rabbit (*Lepus californicus*) from San Andreas, California. A mixed infection of the species found in the domestic rabbit (Kessel and Jankiewicz, 1931) was present. Oocysts of *E. stiedae*, *E. perforans*, and *E. magna,* and probably *E. media,* were recognized. The oocyst shown in plate 18, figure 7, resembles *E. media* more closely than any of the other species. Nieschulz (1923) found oocysts in the intestine and liver of hares which differed from *E. stiedae*. They measured from 26 to 36 μ in length and from 13 to 20 μ in width. These measurements agree with those of the oocysts found in the jack rabbit, which have, however, been identified as the same as those of the domestic rabbit.

COCCIDIA OF THE MOLE

Two moles (*Scapanus latimanus*) both from Berkeley, California, have been examined. In one no coccidia were observed, but in the other large numbers were found. In this latter animal three types of oocysts were seen, those of a small and a large *Eimeria,* and those of what was probably *Cyclospora caryolytica*. Of these the oocysts of the

small *Eimeria* were found in the greatest number, only a few of each of the others being encountered, so this form will be described first.

Oocysts of *Eimeria scapani* sp. nov. (pl. 18, fig. 12) are subspherical in shape; the length of the oocyst varied from 16.0 to 22.4 μ and the width from 14.4 to 16.0 μ; the average size was 19.2 $\mu \times$ 16.0 μ. No micropyle is visible. In five days sporulation was complete. No residual body is found in the oocyst, although several small granules are present. The residual material in the sporocyst consists of numerous large granules.

Only a few oocysts of the large form were found. They measured from 28.0 to 30.0 μ in length and from 22.4 to 25.6 μ in width. The numbers were insufficient to ascertain whether or not this represented a different species.

Oocysts of the third type (pl. 18, fig. 11) were about from 16.0 to 19.2 μ in length and from 12.8 to 16.0 μ in width, and were very few in numbers. By the fifth day they contained two sporoblasts, but none were found then or later in which the sporozoites could be detected. So that it can only be surmised that these represent oocysts of Schaudinn's species *Cyclospora caryolytica*, the mature oocysts of which contain two sporocysts, each with two sporozoites. Schaudinn's description of the life-cycle of this form (see Wenyon, 1926) differs rather markedly from that of other coccidia. He describes a differentiation in the process of schizogony, one line destined to become microgametocytes and the other macrogametocytes. Reichenow has expressed the opinion that this may represent a double infection.

It is unfortunate that the tissue from the mole above described could not be examined, as this might have shed some light on this problem.

EIMERIA OF THE GRAY SQUIRREL

In one gray squirrel, *Sciuris griseus griseus,* from Skaggs, California, very large numbers of oocysts were found in the caecal contents; the intestinal contents were not examined. It was unfortunate that the author was unable to examine the intestinal tract for pathological changes in this case. The oocysts were egg-shaped and a distinct micropyle was present. The cyst wall was very thin in the region of this opening. The oocysts varied in length from 22.4 to 32.0 μ and in width from 16.0 to 19.2 μ; the average size was 28.8 $\mu \times$ 19.2 μ. Almost all the oocysts were compeltely sporulated in two days. The material was lost before a photomicrograph could be taken.

Galli-Valerio (1922) described a new species of coccidia from *Sciurius vulgaris* var. *alpina* as *Eimeria sciurorum*. The average size was 24 μ × 15 μ, and there was a very small micropyle. Sheather (1923), who had not seen Galli-Valerio's report, saw a very few (11) oocysts in a gray squirrel in England; these ranged in length from 21 to 25 μ and in width from 12 to 16 μ. His microphotograph of this form proves it to be identical with the form seen in the California gray squirrel. It seems possible that these may be different from the species described by Galli-Valerio, although there is not sufficient evidence at this time to definitely determine this point.

EIMERIA OF THE SHREW

Oocysts of *Eimeria soricis* sp. nov. (pl. 18, fig. 10) were found in a shrew, *Sorex californicus*, from Berkeley, California. The oocysts found in the intestinal contents were not very numerous. The length of the oocysts varied from 19.2 to 22.4 μ and the width from 12.8 to 14.4 μ; the average size was 19.2 μ × 14.4 μ. The oocysts were ovoidal in shape and the cyst wall, which was relatively thin, was colorless. No micropyle was observed and the cyst wall was of a uniform thickness throughout. Complete sporulation occured in from seven to eight days. A small granule was usually present in the cyst and residual material in the sporocyst, but no residual material in the oocyst.

EIMERIA OF THE WOOD RAT

Twenty-three wood rats (*Neotoma fuscipes*) have been examined for coccidia through the kindness of Miss Fae Donat. Of this number eight were positive for coccidia and fifteen negative. All the wood rats were captured in the vicinity of Berkeley, California. Two species of coccidia were found in these animals.

Eimeria neotomae sp. nov.

Oocysts of *Eimeria neotomae* sp. nov. (pl. 18, figs. 8–9) were found in all the eight wood rats that were positive for coccidia; in four animals *E. neotomae* alone was present. The oocysts of *E. neotomae* varied in length from 16.0 to 22.4 μ and in width from 12.8 to 19.2 μ; the average size was 22.4 μ × 16.0 μ. The oocysts are ellipsoidal in shape; the cyst wall is transparent and smooth; sometimes a small micropyle is visible. Sporulation is completed in a very short time,

thirty-six to forty-eight hours in most cases being sufficient for complete sporulation. The mature oocyst is striking in appearance, as the sporocysts just fill the space in the interior of the oocyst without overlapping. The sporocyst is subspherical in shape and measures about $7.5 \mu \times 6.8 \mu$. The wall of the sporocyst is extremely thin and is seen only with difficulty. Usually there are no granules present in the oocyst following sporulation, as in most species, although in occasional oocysts a granule is seen.

Eimeria residua sp. nov.

Oocysts of *Eimeria residua* sp. nov. (pl. 18, figs. 13–14) were found in four of the eight wood rats infected with coccidia, but always accompanied by *E. neotomae*. The characteristics of the oocysts, however, were so different from those of *E. neotomae*, that there was no difficulty in differentiating between the two species. The oocysts varied in length from 22.4 to 28.8 μ and in width from 19.2 to 25.6 μ; the average size was $25.6 \mu \times 22.4 \mu$. The oocysts of *Eimeria residua* are subspherical in shape. The cyst wall is comparatively thick; it is made up of two layers (Henry, 1932a), the outer layer being brown in color and very rough. It resembles the wall of *E. scabra* of the pig (Henry, 1931). The inner wall is clear and transparent.

Sporulation is a much longer process than in *E. neotomae*. Eight or nine days are required before complete sporulation has occurred. About the seventh day sporoblasts are formed; at this time one or more small granules are found inside the oocyst wall. After the formation of the sporocysts a large residual body (about 6 μ in diameter) is found in the oocyst. This is always clear, homogeneous material; granular residual bodies have not been seen. The sporocysts measure about $10.0 \mu \times 7.5 \mu$; they are pointed at one end.

COCCIDIA OF THE CAT

Davis and Reich (1924) have reported the finding of oocysts in cats and dogs in California. They found two cases of *Isospora bigemina* in dogs and two cases in cats.

Cats and kittens, for the most part raised in the animal house of the Department of Zoology, University of California, have been examined from time to time. They have been found to harbor an infection of *I. felis* almost constantly. In addition to this both, *I. rivolta* and *I. bigemina* have been seen. As far as the author has been able

to ascertain *I. felis* and *I. rivolta* have not been previously reported from California.

Several outbreaks of coccidiosis with subsequent death of the kittens have occurred in this colony. An especially severe outbreak occurred when most of the young cats had, for experimental purposes, been placed on an extremely poor diet, so no conclusions can be drawn as to the pathogenicity of the coccidia.

In addition to the animals which have been listed above, a few other animals have been examined, but no coccidia were found. A list of these animals is given below:

Number examined	Animal	Locality
1	Erethizon epixanthum	Eldorado County, California
1	Reithrodontomys megalotis longicaudus	Berkeley, California
1	Unidentified bat	Berkeley, California
1	Corynorhinus rafinesquii townsendii	Gualala, California
1	Peromyscus maniculatus gambeli	Berkeley, California
2	Peromyscus californicus californicus	Berkeley, California
6	Perognathus sp.	Berkeley, California
1	Perognathus amplus	Arizona
1	Citellus beldingi	Cisco, California
1	Eutanuas quadrimaculatus	Nevada City, California
1	Marmota fluviventer	Amador County, California

Labbé (1893) described a new species from the bat (*Rhinolophus fer-equinum*) as *C. viride*. Lavier (1924) found oocysts in *Rhinolophus hipposideros*, which he believed were different from those described by Labbé and which he named *E. hessei.*

Galli-Valerio (1923) reported a new species in *Arctomys marmota* as *E. marmotae.* Fish (1930) described another new species as *E. monacis*, from *Marmota monax.*

SUMMARY

1. The following new species have been described: *E. beecheyi* from *Citellus beecheyi, E. callospermophili* and *E. bilamellata* from *Callospermophilus chrysodeirus, E. soricis* from *Sorex californicus, E. scapani* from *Scapanus latimanus*, and *E. neotomae* and *E. residua* from *Neotoma fuscipes.*

2. Previously described coccidia from the jack rabbit, the gray squirrel, and two species from cats are reported for the first time from California.

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EXPLANATION OF PLATES

PLATE 17

Figs. 1, 2, 5. *Eimeria bilamellata* sp. nov. from *Callospermophilus chrysodeirus*

Fig. 1. Non-sporulated oocyst. Cytoplasm rounding up in center.

Fig. 2. Sporulated oocyst.

Fig. 5. Oocyst in which the outer, roughened wall is split in half and lies above and below the oocyst. The inner wall is smooth and transparent.

Figs. 3, 4. *Eimeria callospermophili* sp. nov. from *Callospermophilus chrysodeirus*

Fig. 3. Non-sporulated oocyst. Large polar granule and roughened wall can be seen.

Fig. 4. Sporulated oocyst. The residual body is above two sporocysts; other sporocysts not in focus.
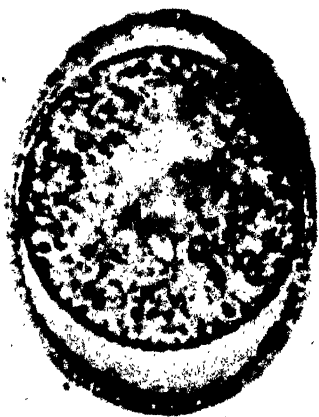
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PLATE 18

Fig. 6. *Eimeria beecheyi* sp. nov. from *Citellus beecheyi.*

Fig. 6. Fully sporulated oocyst. Polar granule at top of oocyst.

Fig. 7. *Eimeria* sp. from *Lepus californicus*

Fig. 7. Sporulated oocyst. Debris clinging to lower end.

Figs. 8, 9. *Eimeria neotomae* sp. nov. from *Neotoma fuscipes.*

Fig. 8. Non-sporulated oocyst.
Fig. 9. Sporulated oocyst, showing nearly round sporocysts.

Fig. 10. *Eimeria soricis* sp. nov. from *Sorex californicus*

Fig. 10. Fully sporulated oocyst.

Figs. 11, 12. Coccidia from *Scapanus latimanus.*

Fig. 11. Oocyst showing two sporoblasts; may be that of *Cyclospora caryolytica* Schaudinn.
Fig. 12. Mature oocyst of *Eimeria scapani* sp. nov. showing pointed sporocysts with large residual granules.

Figs. 13, 14. *Eimeria residua* sp. nov. from *Neotoma fuscipes.*

Fig. 13. Fully sporulated oocyst. Three sporocysts in focus, one out of focus; residual body lying at right of the latter sporocyst. Polar granule at top of oocyst.
Fig. 14. High focus of same oocyst, showing rough oocyst wall.

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ISOSPORA BUTEONIS SP. NOV. FROM THE HAWK AND OWL, AND NOTES ON ISOSPORA LACAZII (LABBÉ) IN BIRDS

BY

DORA PRIAULX HENRY

ISOSPORA BUTEONIS SP. NOV. FROM THE HAWK AND OWL, AND NOTES ON ISOSPORA LACAZII (LABBÉ) IN BIRDS

BY

DORA PRIAULX HENRY

Since Labbé (1893a) named the *Isospora* of birds, *Isospora lacazii* (he placed this form in the genus *Diplospora*, which is a synonym for the genus *Isospora*), there has been some question as to whether the *Isospora* found in all birds represented one species. In fact, Labbé (1893a) described two species in birds, *I. lacazii* from the lark and gold finch, and *I. rivoltae* from the finch, shrike, and titmouse. The cysts of *I. lacazii* measured from 23 to 25 μ, while those of *I. rivoltae* measured only from 16 to 18 μ, the cyst wall was thinner, and the sporulation time was much longer. In 1896, however, Labbé reported *Isospora* from other birds in which he had found oocysts of intermediate size, and he had also noticed great variation in thickness of the cyst wall and in the sporulation time, so that he did not think it possible to separate *I. lacazii* into different species. Therefore he abandoned the species *Isospora rivoltae*.

The coccidium of birds was given the specific name *lacazii* by Labbé in 1893 and he referred to it as such in a second paper in 1893. In papers dated 1894, 1896, and 1899 *lacazei* is used and this form has been used by most authors since that time. However, *lacazii* is correct as it was used when the species was named. Wenyon (1926) cites as the paper in which Labbé named this form a second paper (1893b) in which Labbé named a new species of marine birds, *Coccidium roscoviense*. This latter species Wenyon considered Labbé as having described in 1894.

Later workers studying *Isospora lacazii* have suspected that more than one species was represented, as they found great variation in the size of the oocysts. Recently Boughton (1930) using biometric constants has found great variation in the size of the oocysts of the English sparrow and also that they vary significantly in a single host.

He reports large oocysts at the beginning of an infection followed by smaller ones. Correlated with the appearance of the smaller oocysts was an increase in production of the oocysts. Kartchner and Becker (1930) using the same methods in following the course of infection in ground squirrels found variations in the size of the oocysts from day to day, but did not find diminution in the size of the oocysts as the infection progressed. Boughton reaches the conclusion that only one species, *Isospora lacazii*, is represented.

In an effort to reconcile these conflicting reports a study has been made of the coccidia found in a miscellaneous group of birds; the results of these examinations are presented below.

TABLE 1

BIRDS OTHER THAN PASSERINES EXAMINED

Bird	Number Examined	Positive for Isospora	Negative for Isospora
Oxyechus vociferus	4	1	3
Totanus melanoleucus	1	....	1
Larus argentatus	1	....	1
Chlidonias nigra	1	....	1
Zenaidura macroura	3	....	3
Colaptes cafer	4	4	....
Balanosphyra formicivora	2	....	2
Cathartes aura	1	....	1
Speotyto cunicularia	2	....	2
Asio flammeus	1	1	....
Accipiter cooperii	1	1	....
Buteo borealis	2	2	....
Buteo swainsoni	1	1	....
Falco sparverius	4	....	4

The author wishes to acknowledge the continued interest of Dr. C. A. Kofoid in this work, the cooperation of Dr. Alden H. Miller in identifying many of the birds, and of Mr. O. L. Williams for many specimens.

One hundred and sixty seven birds were examined for coccidia. An endeavor was made to collect as many different species as possible and from different sections of California. For the most part, not more than one bird of a kind was taken from any one region. Besides the birds that were shot, a few live birds, mainly young ones, were used for experimental purposes.

Of the 167 birds examined, 140 belonged to the order Passiformes, and 27 to several other orders. It does not seem necessary to list the different species of the passerines in which coccidia were found, but

it may be of interest to list the others (see table 1). This list includes several of the water birds in which Labbé (1893*b*) found *Coccidium roscoviense,* but neither this species nor *Isospora lacazii* was found. Fifty per cent of the passerines were positive for *Isospora lacazii,* while only 33 per cent of the other species were infected. The total number in the latter group was so small, however, that this percentage is probably not valid. Wasielewski (1904) found 20 per cent of the 400 birds which he examined positive for coccidia. Boughton (1929) found 66 per cent of the 95 English sparrows examined positive for *Isospora lacazii.*

In this study of the *Isospora* found in birds the author has found, with one exception to be considered later, only *Isospora lacazii.* Variations in size of the oocysts were found comparable to those described by previous authors. The oocysts varied in length from 16.0 to 32.0 μ and in width from 12.8 to 28.8 μ. In any one bird the widest range in length of oocysts was from 22.4 to 32.0 μ and in width from 19.2 to 25.6 μ. More blackbirds were examined than any other group and it was found that in some blackbirds the large oocysts predominated, while in others the mean dimensions were considerably smaller. In some birds oocysts which varied greatly in size were present, while in others the size range was less.

The oocysts of *I. lacazii* (pl. 19, figs. 5–8) are often subspherical in shape, but frequently oval or ovoidal forms are encountered. Usually the micropyle is inconspicuous or cannot be seen at all. The wall of the oocysts from the flicker, *Colaptes cafer,* and the sparrow, *Passer domesticus* (the only cysts examined for this characteristic), is made up of three layers. There is an outer and innermost thin layer, whereas the intermediate layer is comparatively thicker. It has been impossible to separate these layers and they were seen only in oocysts which had been kept for some time and which were partly collapsed. Complex walls are quite common in the genus *Eimeria* (see Henry, 1932*a*). Oocysts of *Isospora belli* from man (see Wenyon, 1926) also show a complex wall. In this case there is an outer thick layer showing a double contour and an inner and much finer membrane.

Sporulation of the oocysts of *I. lacazii* is very rapid; twenty-four hours is usually sufficient time for complete development. One or a few granules are usually present in the oocyst after sporulation. The sporocysts usually contain a rather large residual body and frequently a knob at one end of the sporocyst is prominent (pl. 19, fig. 7).

The wall of the mature oocyst collapses readily and as a result almost all oocysts which have been stored for any length of time have collapsed walls.

Several young birds were kept in the laboratory for study. Of these the most interesting were four flickers (*Colaptes cafer*) which were taken from the nest. Comparisons with skins showed that they were between seven and ten days old at the time they were taken. All four birds were heavily infected with *Isospora lacazii*. Two died three days later and in both many cysts were found, particularly in the duodenum. A third flicker died eight days after being taken from the nest, but the last cysts were found two days before death. The fourth flicker lived for eight months. The last oocysts were found seven days after the birds were brought to the laboratory and, although examinations were made periodically, no oocysts were found after this time. The oocysts from these birds were subspherical in shape and comparatively small. The range in length was from 19.2 to 25.6 μ and in width from 19.2 to 22.4 μ.

One sparrow (*Passer domesticus*) which was just learning to fly was brought to the laboratory. Many oocysts of *I. lacazii* were found. Each dropping was examined and measurements made of the oocysts. The sparrow died after six days, but an examination of the intestine showed no lesions that could be attributed to coccidiosis. In some droppings no oocysts were found, although several examinations were made. There was very little variation in the size of the oocysts over the six-day period. The oocysts in this bird were comparatively larger than those of the flickers. They measured from 22.4 to 32.0 μ in length and from 19.2 to 25.6 μ in width; the greater number were from 25.6 to 32.0 μ long. These oocysts were mostly ovoidal instead of subspherical, as in the flickers.

Two young white-crowned sparrows (*Zonotrichia leucophrys*) were caught at different times. Both of these were negative for coccidia. A young robin (*Turdus migratorius*), which died the day following its capture, contained many oocysts of *I. lacazii*. It was probably injured in being captured. An adult robin and an adult towhee (*Pipilo maculatus falcifer*) were kept in the laboratory for several days; both of these were heavily infected.

From the above study of the coccidia of birds one must draw the conclusion that only one species, *Isospora lacazii*, is represented. At least, with our present methods of differentiation of species, no other conclusion is possible. If experiments could be carried out with these

species as easily as with chickens, our knowledge of this form might be extended. With the exception of the species to be described below, the oocysts of only one bird seemed to offer the possibility of a different species. Unfortunately, only a few oocysts were found in this bird (*Oxyechus vociferus vociferus*). They corresponded in size to those of Labbé's abandoned species, *I. rivoltae*. Three other killdeers were examined, but were negative.

A new species was encountered, however, in four hawks and later in an owl. There was no hesitation in differentiating it from *I. lacazii*. A description of this species follows.

Isospora buteonis sp. nov.

Oocysts of *Isospora buteonis* sp. nov. (pl. 19, figs. 1–4) were found in the intestinal contents of several hawks; in two *Buteo borealis* from the same region (Mendocino County, California), but collected five months apart, in one *Buteo swainsoni* (Modoc County, California), and in one *Accipiter cooperii* (Alameda County, California). Four sparrow hawks (*Falco sparverius*) from Mendocino County, California, were also examined, but no oocysts were found.

These oocysts differed strikingly from those of *I. lacazii*. They were smaller; the oocyst was more irregular in shape; the wall was thinner and more fragile; and completely sporulated oocysts were found in the intestine.

The oocysts measure from 16.0 to 19.2 μ in length and from 12.8 to 16.0 μ in width. The oocyst wall (pl. 19, fig. 2) is extremely thin; it encloses the sporocysts very tightly; in fact, it can only be detected with difficulty except between the two sporocysts. In *I. lacazii*, on the other hand, the wall is almost always separate from the sporocysts. The oocysts of *I. bigemina* of dogs and cats, according to Wenyon (1926), often have this appearance, and the wall of these oocysts is also very thin. In *I. buteonis* the wall is extremely fragile, as very few intact oocysts are seen, even when hundreds of sporocysts are present. It seems unlikely that this results entirely from the technique of making the smear: more probably it occurs regularly in the intestinal contents.

Completely sporulated oocysts were found in the intestinal contents and in scrapings of the intestine. In only one bird were immature forms seen. In these oocysts (pl. 19, fig. 1) there were very granular sporoblasts; the oocyst wall was more easily detected than in the mature oocyst. No earlier stages were seen.

The sporocysts vary in length from 9.6 to 13.0 μ and in width from 8.0 to 10.4 μ. The sporocyst is ovoid in shape, rounded at both ends rather than pointed at one end as in *I. lacazii.* The wall is thicker than that of the oocyst. In it are four sporozoites and the residual material. There is a comparatively large amount of this material, which has no definite form as it has in *I. lacazii,* but it is dispersed throughout the sporocyst. For this reason in many of the sporocysts the sporozoites can only be seen with difficulty. In a few of these very little or no residual material is found. The residual material which is present is in the form of rather large granules.

The sporozoites measure from 5 to 7.8 μ in length and from 1.3 to 2.5 μ in width. They appear to consist of homogeneous material. The sporozoites are rounded at both ends and are almost straight (very slightly crescent-shaped).

Whether or not this form was a parasite of the hawk was questioned when fully sporulated oocysts were found, as, with few exceptions, in warm-blooded hosts the oocysts do not complete their development until they pass out of the host. However, these oocysts were found in cells of the intestinal epithelium when scrapings were made. Also, such large numbers were present in several of the birds that it would seem unlikely that they were the parasite of some insect or mammal that had been ingested. The great divergence in the feeding habits of the species in which this coccidium was encountered also tends to discredit the possibility that these organisms were parasites of animals which served as food. In this respect they resemble *Isospora bigemina* of dogs and cats. Wenyon (1926) describes fully sporulated cysts of this species in the sub-epithelium of the cat intestine.

Oocysts of *I. buteonis* were found for the most part in the duodenum in all of the hawks encountered. It seems likely that further work will show that this species is limited to the duodenum.

Apparently *I. buteonis* does not cause serious injury to hawks. All the birds were shot and with one exception no pathological changes were seen in the intestine. In one bird, the Swainson hawk, some blood-tinged intestinal contents were seen. In this bird the oocysts were comparatively few and there was a heavy infestation of tapeworms. For this reason it seems unlikely that *I. buteonis* is pathogenic.

A few experimental transfers were attempted with both *I. lacazii* and *I. buteonis.* A chicken was fed a massive dose of oocysts of *I. lacazii* from five blackbirds. A dove, *Zenaidura macroura marginella,*

TABLE 2

DISTRIBUTION OF COCCIDIA IN BIRDS

ORDER	HOST	SPECIES OF COCCIDIA	FIRST DESCRIBED
Galliformes	Chicken and California quail	Eimeria tenella	Railliet and Lucet (1891)
	Chicken and California quail	Eimeria acervulina	Tyzzer (1929)
	Chicken and California quail	Eimeria mitis	Tyzzer (1929)
	Chicken and California quail	Eimeria maxima	Tyzzer (1929)
	Turkey	Eimeria meleagridis	Tyzzer (1927)
	Turkey	Eimeria meleagrimitis	Tyzzer (1929)
	Pheasant (Phasianus colchicus torquatus)	Eimeria phasiani	Tyzzer (1929)
	Quail (Colinus virginianus)	Eimeria dispersa	Tyzzer (1929)
Columbiformes	Pigeon	Eimeria pfeifferi	Labbé (1896)
Anseriformes	Geese (kidneys)	Eimeria truncata*	Railliet and Lucet (1891)
Passeriformes and a few other orders	Song birds, etc.	Isospora lacazei	Labbé (1893a)
	Song birds, etc.	Isospora rivoltae	Labbé (1893a)
	Song birds, etc.	Eimeria roscoviense*	Labbé (1893b)
Charadriiformes			
Gruiformes	Fulica atra and Gallinula chloropus	Jarrina paludosa	Léger and Hesse (1922)
Falconiformes and Strigiformes	Hawk and owl	Isospora buteonis	Henry (1932)

* May belong to genus Jarrina (Léger and Hesse, 1922).

which was negative for coccidia when captured, was fed oocysts from the English sparrow, and a young white crowned sparrow was fed oocysts from the robin. None of these birds became infected.

A mouse and one of the young flickers, after it had failed to pass oocysts of *I. lacazii* for four days, were fed large numbers of oocysts of *I. buteonis.* Neither became infected. The above experiments, although meager, bear out the prevailing ideas of host-parasite specificity.

An owl (*Asio flammeus*) from the vicinity of Seattle, Washington, has been examined through the courtesy of Dr. Erna Gunther, of the Washington State Museum (University of Washington). The duodenum was filled with oocysts of *I. buteonis,* which agreed in all respects with those found in hawks. This species is therefore not so restricted in habitat as was at first thought. Two owls (*Speotyto cunicularia*) had been examined in California, but no oocysts were seen.

The following table, compiled from various sources, is an attempt to summarize the species of coccidia from birds which have been described. This table draws attention to the rather striking fact that in the case of the order Passiformes and other rather closely related orders only a single species of coccidium is involved, whereas the species included in the order Galliformes have several coccidial parasites, most of which show rather strict host specificity. In this respect this latter order closely resembles the condition encountered in mammals.

SUMMARY

1. An examination of a miscellaneous group of wild birds has convinced the author that *I. lacazii* as described by Labbé includes but a single species.

2. Measurements of oocysts from individual birds during the course of a single infection have failed to confirm Boughton's findings in regard to a progressive diminution in size of the oocysts.

3. A new species of coccidium, *Isospora buteonis,* from the hawk and owl, is described, the oocysts of which differ greatly from those of *Isospora lacazii.*

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EXPLANATION OF PLATES

PLATE 19

Figs 1–4   Oocysts of *Isospora buteonis* sp nov

Fig 1   Immature oocyst found in scrapings of duodenum   Unstained

Fig 2   Mature oocyst   Wall visible only between sporocysts   Sporozoites covered by residual material

Fig 3   Mature sporocyst, sporozoites partially seen

Fig 4   Injured sporocyst, pointed at one end because of collapse   Sporozoites seen

Figs 5–8   Oocysts of *Isospora lacazii* (Labbé), figs 5–7 from robin; fig 8 from flicker

Fig 5   Non sporulated oocyst

Fig 6   Oocyst beginning to sporulate, 2 sporoblasts formed

Fig 7   Completely sporulated oocyst, figs 6 and 7, same oocyst

Fig 8   Fully sporulated oocyst   Notice the difference in shape from the above oocyst
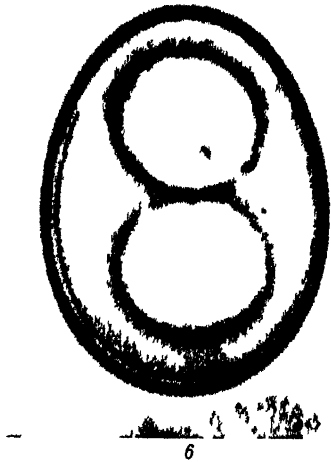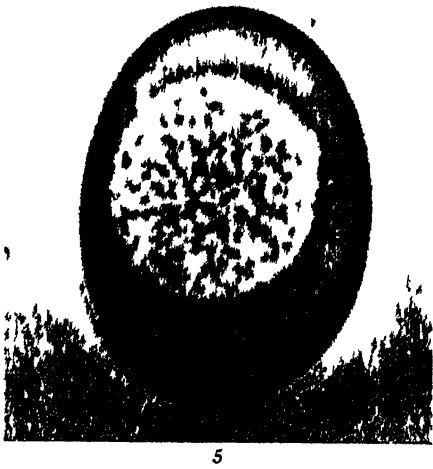
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CONTRIBUTIONS TO A KNOWLEDGE OF THE MYSIDACEA OF CALIFORNIA

I. ON A COLLECTION OF MYSIDAE FROM LA JOLLA, CALIFORNIA

BY

W. M. TATTERSALL

CONTRIBUTIONS TO A KNOWLEDGE OF THE MYSIDACEA OF CALIFORNIA

I. ON A COLLECTION OF MYSIDAE FROM LA JOLLA, CALIFORNIA

BY

W. M. TATTERSALL

The four species of Mysidae here reported upon, one of which is new to science, were picked out from surface tow-nettings taken from the pier at La Jolla, California, by the authorities of the Scripps Institution, over a period of three years, 1916–1918. I am greatly indebted to the late Dr. C. O. Esterly and to Dr. T. W. Vaughan, Director of the Scripps Institution, for the opportunity of examining and reporting on the collection. My study has enabled me to amplify the earlier descriptions of two other species and to clear up some doubtful points of structure and affinity.

The known Mysidacean fauna of California is not extensive. The first species to be recorded from its shores was *Callomysis maculata*, described by Holmes in 1895 from Trinidad, California. In 1896, the same author described *Neomysis mercedis* from Lake Merced, California. In 1900, Holmes published his synopsis of the Stalk-eyed Crustacea of California in which, in addition to the two species already mentioned, he included and described the following new species: *Mysis costata*, *Neomysis franciscorum*, *Heteromysis spinosus*, *Mysidopsis elongata*, and *Siriella pacifica*—all from the coast of California. In 1913 Hansen redescribed *Mysis costata*, *Neomysis franciscorum*, and *Siriella pacifica* from an examination of the type specimens. In 1914 Esterly recorded *Holmesiella anomala, Pseudomma* sp., and *Mysis costata* from the San Diego region but gave no details nor data of his specimens. No papers on Californian Mysidacea have appeared since 1914 and no other species are known from its coasts. Various papers on Pacific oceanic forms by Colosi, Faxon, Hansen, Ortmann, and Zimmer, include species captured off the coast of California but over deep water, and belonging therefore to the purely oceanic fauna of the Pacific and not to the littoral or coastal fauna

of California. Excluding all these forms, the Mysidacean fauna of
California includes the following species:

Siriella pacifica Holmes   *Mysis costata* Holmes
Callomysis maculata Holmes   *Neomysis mercedis* Holmes
Holmesiella anomala Ortmann   *Neomysis franciscorum* Holmes
Pseudomma sp.   *Heteromysis spinosus* Holmes
Mysidopsis elongata Holmes

In this paper I record three of these species, *Siriella pacifica Archaeo-
mysis maculata* (=*Callomysis maculata*), and *Mysidopsis elongata*
and add a new species, *Mysidopsis californica.*

In the accompanying paper I present a report on the Mysidacea
collected during the survey of San Francisco Bay by the "Albatross"
in 1914 and there deal with five species of the genus *Neomysis*, three
of which are already known from Californian waters, one, *N. kadi-
akensis* Ortmann, known hitherto only from the Pacific coast of Alaska,
and one new species. The total number of species of Mysidacea now
known from California is therefore twelve.

Of the species here recorded from La Jolla, *Siriella pacifica,
Mysidopsis californica,* and *Archaeomysis maculata* are peculiar to
California, while the fourth species, *Mysidopsis elongata,* is also
recorded from the more southern waters off Callao. None of these
species is included in the fauna of San Francisco Bay. In fact the
entire dissimilarity between the two collections is rather striking; the
one from La Jolla has a distinctly southern facies, while that from
San Francisco Bay has a pronouncedly northern one.

Suborder MYSIDA
Family MYSIDAE Dana
Subfamily SIRIELLINAE Norman
Genus SIRIELLA Dana

Siriella pacifica Holmes

S. pacifica Holmes, 1900, p. 227.
S. pacifica Hansen, 1913, p. 175, pl. 9, figs. 1 *a–f.*

Locality.—La Jolla, California.
Haul 432, apparatus 12, 10.3.16, 12 midnight, one adult male, 10 mm.
Haul 2308, apparatus 9, 8.23.17, 12 midnight, one male 7 mm., two im-
                        mature females, 6–8 mm.
Haul 2394, apparatus 9, 9.10.17, 12 midnight, one adult male, 10 mm.
Haul 2425, apparatus 9, 9.15.17, 4 a.m., one adult female, 10 mm.
Haul 3005, apparatus 9, 1.8.18, 11:45 p.m., one adult female, 12 mm.
  Remarks.—I have nothing to add to Hansen's redescription of this
species. These specimens are in complete agreement with his account.
  Distribution.—Holmes' types were found at San Diego. The
species is only known from the coasts of California.

SUBFAMILY GASTROSACCINAE Norman

Genus ARCHAEOMYSIS Czerniavsky, 1882

Genus *Callomysis* Holmes, 1895

The genus *Archaeomysis* was defined in 1882 by Czerniavsky to include the species *A. grebnitzkii,* found in the stomach of a gadoid fish caught off Bering Island. Its most salient feature is the biramous nature of the pleopods in both sexes. Czerniavsky realized the importance of this character and, as is implied by the name which he gave to the genus, rightly regarded it as primitive.

The genus *Callomysis* was formed by Holmes in 1895 for a Californian species, *C. maculata.* There is nothing in Holmes's diagnosis to distinguish the genus from *Archaeomysis* and I agree with the opinion, already tentatively expressed by Hansen (1910), that the two genera are really the same. It is true that Czerniavsky in his description of *A. grebnitzkii* states that it is the inner ramus of the pleopods of the female which is rudimentary, while Holmes in his diagnosis of *Callomysis* says that the outer ramus is smaller than the inner in these appendages. This is, however. merely a diversity of interpretation rather than an actual difference in fact. My own opinion inclines to the view that Holmes has correctly interpreted the morphology of the female pleopods. Otherwise there is the closest agreement between the two genera and I have no hesitation in regarding them as identical.

Hansen has referred the genera *Archaeomysis* and *Callomysis* to the subfamily Gastrosaccinae and there is no doubt to my mind, that this represents their true affinity. Czerniavsky founded a special subfamily *Archaeomysinae* to include *Archaeomysis,* thereby emphasizing the primitive character of the pleopods. The genus, however, is otherwise so closely allied to *Gastrosaccus* and agrees with it so minutely in detail in all other characters that Hansen's view of its systematic position is clearly the more natural and acceptable.

Archaeomysis maculata Holmes

Figures 1-13

Callomysis maculata Holmes, 1895, p. 582, pl. 21, figs. 37-44.
Callomysis maculata Holmes, 1900, p. 224.

Locality.—La Jolla, California.

Haul   137, apparatus 12, 13.9.16, 12 midnight, one immature male,
       6 mm.
Haul   361, apparatus 12, 25.9.16, 8 p.m., one young specimen, 4 mm.
Haul   432, apparatus 12, 3.10.16, 12 midnight, one adult female, 12
       mm., and one young, 4 mm.
Haul 1697, apparatus   9, 2.4.17, 4 a.m., one adult female, 11 mm.
Haul 1720, apparatus   9, 26.4.17, 12 midnight, one male, 7 mm.
Haul 1789, apparatus   9, 10.5.17, 8 p.m., one male, 8 mm.; one female,
       10 mm.; one young, 6 mm.
Haul 1966, apparatus 12, 16.6.17, 12 midnight, two young, 5 mm , and
       5.5 mm.
Haul 3005, apparatus   9, 8.1.18, two young males, 7 mm.

Length.—Adult female, 12 mm.

Description.—Carapace produced in front into a moderately long, acutely pointed rostral projection, extending anteriorly to the distal margin of the eyes; posterior dorsal margin of the carapace without a fringe of lappets but with two simple digitate lobes, not forwardly directed.

Fifth abdominal somite with the median dorsal posterior margin drawn out into a laterally compressed acute spine overlapping the sixth somite for about one-quarter of its length. Sixth abdominal somite one and a half times as long as the fifth.

Antennular peduncle with two spines on the outer margin of the second joint. Antennal peduncle as long as the first two joints of the antennular peduncle; distal joint quite short. Antennal scale shorter than its peduncle, slightly longer than the first joint of the antennular peduncle, four times as long as broad. with a distinct distal joint marked off by an articulation; outer margin entire and terminating in a very pronounced spine projecting well beyond the apex of the scale. Labrum produced into a well marked forwardly projecting spine

Sixth joint of the endopod of the third to the eighth thoracic limbs divided into from eight (in the third) to twelve (in the eighth) subjoints; expanded basal plate of the exopods of the thoracic limbs with two small spines on the outer distal corner in the second to the seventh pairs, rounded in the first and last.

Telson as long as the sixth abdominal somite, two and a quarter times as long as broad at the base; cleft for one-sixth of its length, the cleft armed with serrations; lateral margins armed with nine or

ten spines; each lobe of the apex armed with two closely placed, long powerful spines about one-sixth of the telson in length.

Uropods subequal in length, one-sixth longer than the telson; inner uropod with eight rather stout, widely spaced spines on the inner
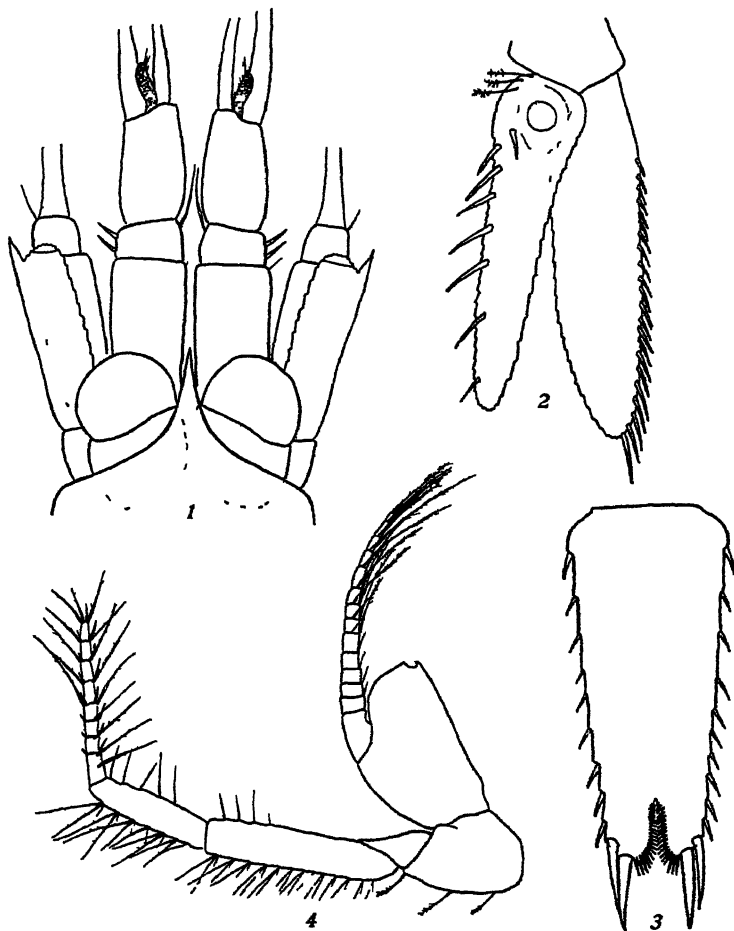
Figs. 1–4. *Archaeomysis maculata* Holmes

Fig. 1. Anterior end of a young male showing rostrum, antennules, antennal
         scale, and peduncle. × 78.
Fig. 2. Uropods. × 78.
Fig. 3. Telson. × 78.
Fig. 4. Third thoracic limb. × 62.

margin; outer uropod with twenty-two closely set spines on the distal three-quarters of the outer margin.

Pleopods of the female all biramous; in the first pair the rami are less than half as long as the peduncle, equal in lentgh, and armed with a few plumose setae; the distal margin of the basal joint armed with several very long plumose setae; in the remaining pairs the exopod is a very minute lobe tipped with two setae, the endopod one-jointed,

longer than the peduncle, and armed with several very long plumose setae.

First pleopod of the male with the peduncle equal in length to the outer ramus and armed with several very long plumose setae on the outer margin; endopod small and one-jointed; exopod five-jointed.
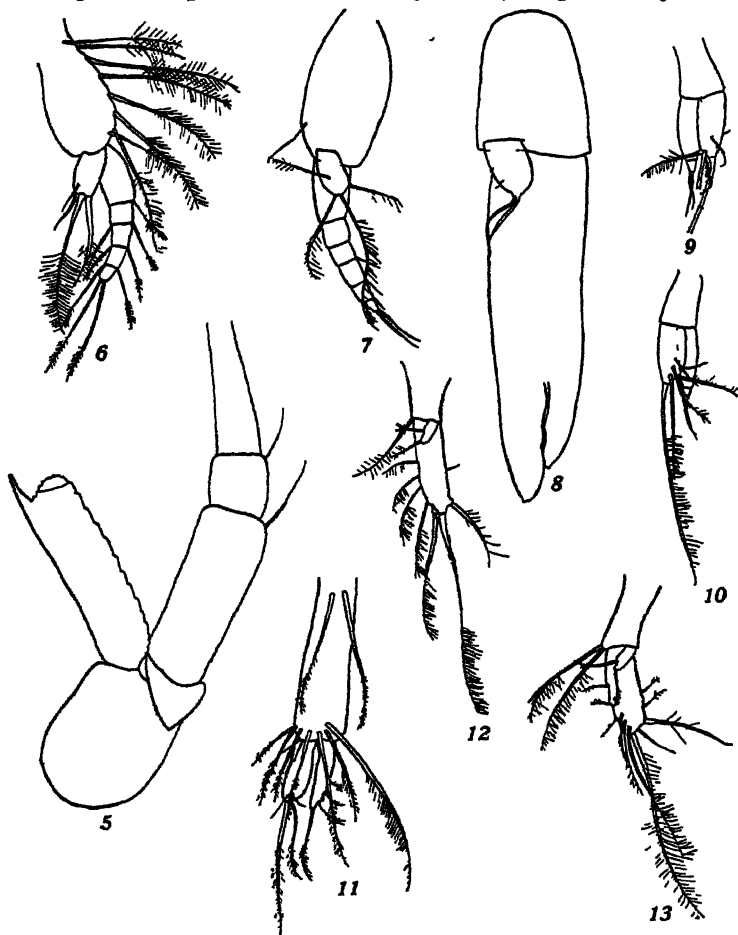
Figs. 5–13. *Archaeomysis maculata* Holmes

Fig. 5. Antennal scale and peduncle. × 78.
Fig. 6. First pleopod of the male. × 100.
Fig. 7. Second pleopod of the male. × 100.
Fig. 8. Third pleopod of the male. × 100.
Fig. 9. Fourth pleopod of the male. × 100.
Fig. 10. Fifth pleopod of the male. × 100.
Fig. 11. First pleopod of the female. × 100.
Fig. 12. Second pleopod of the female. × 100.
Fig. 13. Third pleopod of the female. × 100.

Second pleopod of the male similar to the first but without plumose setae on the outer margin of the peduncle, the outer ramus six-jointed.

Third pleopod of the male (in an immature specimen) with a short

peduncle; endopod single-jointed; exopod greatly developed and divided at the distal end into two digitate lobes.

Fourth and fifth pleopods of the male similar; rami slightly longer than the peduncle; endopod single-jointed; exopod slightly longer than the endopod and three-jointed.

Remarks.—While it is comparatively easy to determine the genus to which the California specimen belongs, it is much more difficult to decide to which species they should be referred. The matter is rendered difficult by the fact that the only males at my command are still not fully grown so that the adult form of the male pleopods is not known to me.

My specimens differ from Holmes's description of *Callomysis maculata* in the following points:

(1) the rostral plate is long and pointed, not short and subtriangular; (2) there is a very prominent dorsal spine on the posterior median dorsal margin of the fifth abdominal somite. Holmes makes no mention of such a spine in the species. In both these respects the California specimens conform to Czerniavsky's description of *Archaeomysis grebnitzkii;* and with the exception of these two points there is very close agreement between Holmes's account of *C. maculata* and Czerniavsky's description of *A. grebnitzkii.* The only real divergence is in the form of the fourth and fifth pleopods of the male. Holmes states that in *C. maculata* the endopod, while shorter than the exopod, is divided into several articulations, while Czerniavsky describes the endopod of these appendages in *A. grebnetzkii* as uni-articulate.

I am unable to compare my specimens with these descriptions because none of my males is adult. I have described and figured the pleopods in detail so that future workers may be able to decide how much the differences may be owing to immaturity. For the moment, therefore, I must regard *Archaeomysis grebnitzkii* and *Callomysis maculata* as separate and distinct species.

Distribution.—Trinidad, California (Holmes).

SUBFAMILY MYSINAE

TRIBE LEPTOMYSINI

Genus MYSIDOPSIS G. O. Sars

Mysidopsis californica, sp. nov.

Figures 14-25

Locality.—Mission Bay (False Bay), California—numerous individuals of both sexes up to 8 mm.

Description.—Carapace produced in front into a very short, bluntly-rounded, triangular plate not covering any part of the eyestalks; anterio-lateral angles rounded.

Sixth segment of the pleon one and a half times as long as the fifth.

Antennular peduncle in the male shorter and stouter than in the female with the last joint relatively shorter and having a well developed setose lobe. Antennal scale extending for one-third of its length beyond the antennular peduncle, narrowly lanceolate in shape and setose all round, nine times as long as broad, distal joint equal to about

one-quarter of the scale, apex rather acute; antennal peduncle about half as long as the scale and slightly shorter than the antennular peduncle; third joint two-thirds of the length of the second.
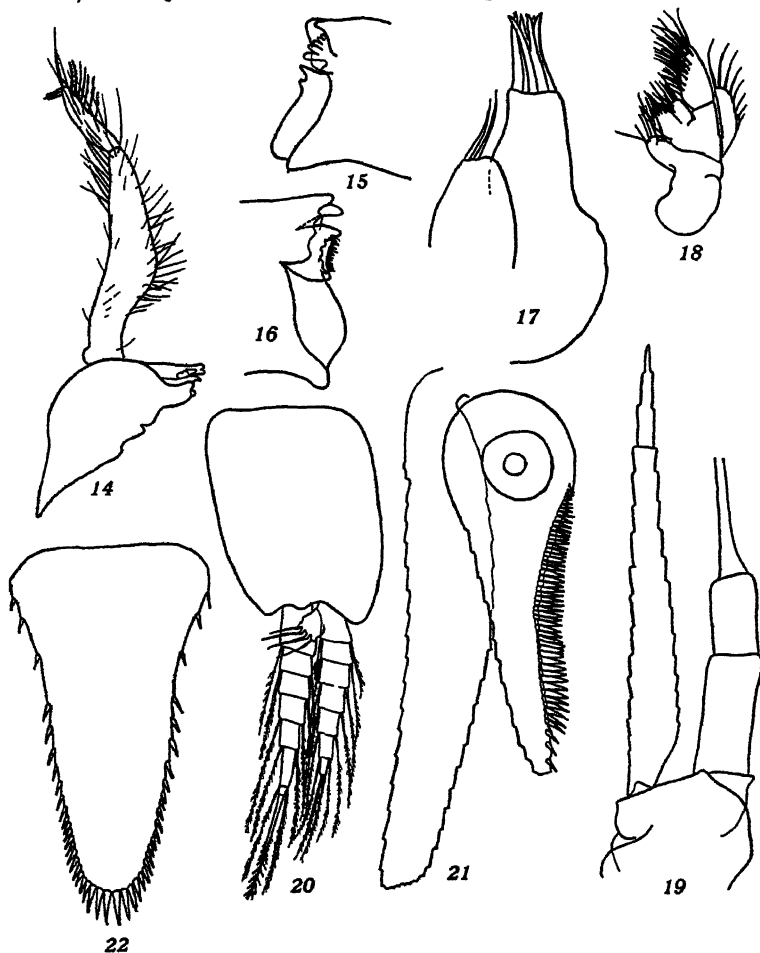
Figs. 14–22. *Mysidopsis californica* sp. nov.
Fig. 14.   Mandible and palp.   × 100.
Figs. 15–16.   Cutting edges of the right and left mandibles.   × 330.
Fig. 17.   First maxilla.   × 330.
Fig. 18.   Second maxilla.   × 100.
Fig. 19.   Antennal scale and peduncle.   × 100.
Fig. 20.   Fourth pleopod of the male.   × 100.
Fig. 21.   Uropods.   × 100.
Fig. 22.   Telson.   × 100.

Eyes large, about one and three-fifths as long as broad; cornea occupying nearly half of the whole eye in dorsal view; pigment black.

Mandibles with a molar process. Maxillulae with the inner lobe small and armed with two setae. Maxillae with a well developed exopod but without the setiferous expansion from the second joint.

Third to the eighth thoracic limbs long and slender; sixth joint divided by a transverse articulation into two subjoints; dactylus long and slender, longer than the distal subjoint of the sixth joint; a small curved finger-like process (gill?) on the outer margin of the basal joint; basal plate of the exopod with the outer distal corner rounded.
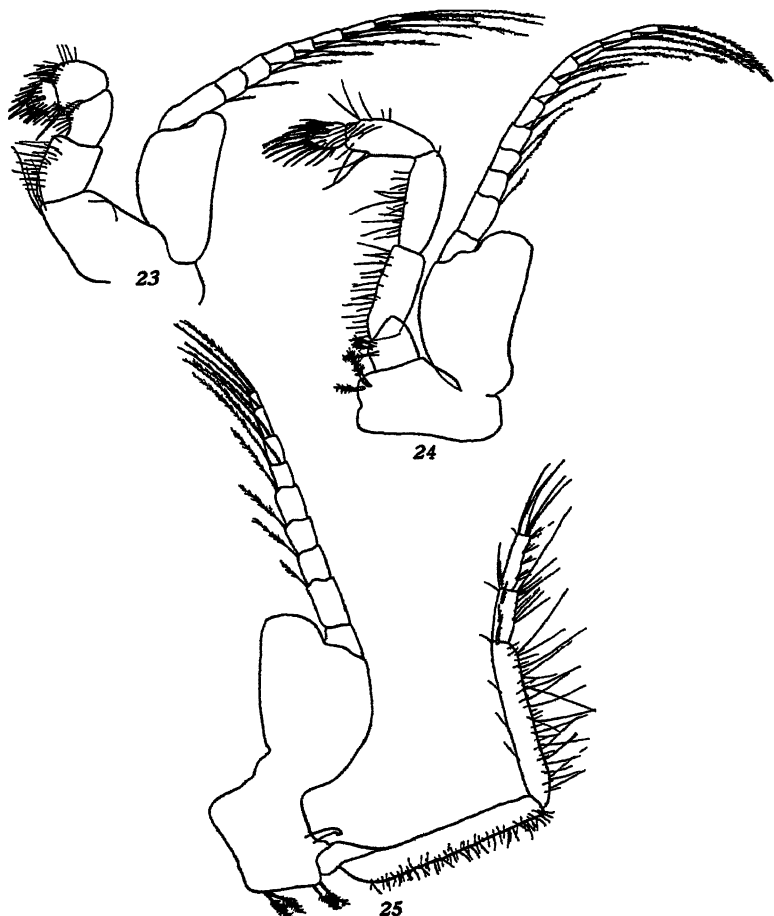
Figs. 23–25. *Mysidopsis californica*, sp. nov.
Fig. 23.   First thoracic limb.   × 100.
Fig. 24.   Second thoracic limb.   × 100.
Fig. 25.   Third thoracic limb.   × 100.

Telson as long as the sixth abdominal somite, one and three-quarters times as long as broad at the base, linguiform in shape, apex entire and rounded; lateral margins armed with about twenty-five spines extending throughout the entire margin; the proximal two-fifths of the margins with three or four distantly placed spines; the distal three-fifths of the margins with the spines crowded together; apical pair of spines about one-tenth of the telson in length; no plumose setae.

Inner uropod about one and one-third as long as the telson; inner margin armed with a dense row of large blunt spines, about fifty in number, extending from the level of the statocyst to the apex. Outer uropod nearly twice as long as the telson.

Pleopods in the male typical for the genus; fourth pair with the exopod slightly longer than the endopod and terminating in a long powerful plumose seta.

Remarks.—This species is distinguished from *M. elongata* described below by the form of the telson and its armature of spines, by the narrow elongate antennal scale, and by the armature of the inner uropod. It also differs in having an exopod on the maxillae and is, in this respect, a more typical member of the genus. Zimmer (1912) created a new genus *Paramysidopsis* to include three species from the West Coast of Africa, which possessed small finger-like processes on the basal joint of the endopod of the thoracic limbs, interpreted by him as gill processes. In 1918 Zimmer canceled the genus on the ground that such processes were not infrequently present in other Mysidacea and could hardly be regarded as of generic value. *Mysidopsis californica* agrees with the West African species in possessing such processes on the third to the eighth thoracic limbs but not on the first and second.

Mysidopsis elongata Holmes

Figures 26–38

M. elongata Holmes, 1900, p. 226, pl. 4, figs. 77–80.
M. pacifica Zimmer, 1918, p. 19, text figs. 16–24.

Locality.—La Jolla, California.

Haul   87, apparatus 12, 29.8.16, 8 p.m., one male and several young.
Haul 137, apparatus 12, 13.9.16, midnight, three males and four females.
Haul 377, apparatus 12, 21.9.16, 4:30 a.m., thirty-three young.
Haul 381, apparatus 12, 25.9.16, 8 p.m., six females, two males, and four young.
Haul 432, apparatus 12, 3.10.16, midnight, five females, three males, and three young.
Haul 586, apparatus 12, 17.10.16, 8 p.m., four males and three females.
Haul 1237, apparatus 9, 13.1.17, 8 p.m., two females, two males, and one young.
Haul 1418, apparatus 9, 21.2.17, 8 p.m., seven males, eight females, and two young.
Haul 1789, apparatus 9, 10.5.17, 8 p.m., one female.
Haul 1846, apparatus 9, 23.5.17, midnight, two females.
Haul 1966, apparatus 12, 16.6.17, midnight, eight males, seven females, and two young.
Haul 2301, apparatus 12, 29.6.17, midnight, three females and one young.
Haul 2308, apparatus 9, 23.8.17, midnight, one female.
Haul 2393, apparatus 9, 9.9.17, 8 p.m., three males, four females, and nine young.
Haul 2913, apparatus 9, 20.12.17, 3:45 a.m., five males, two females, and nine young.
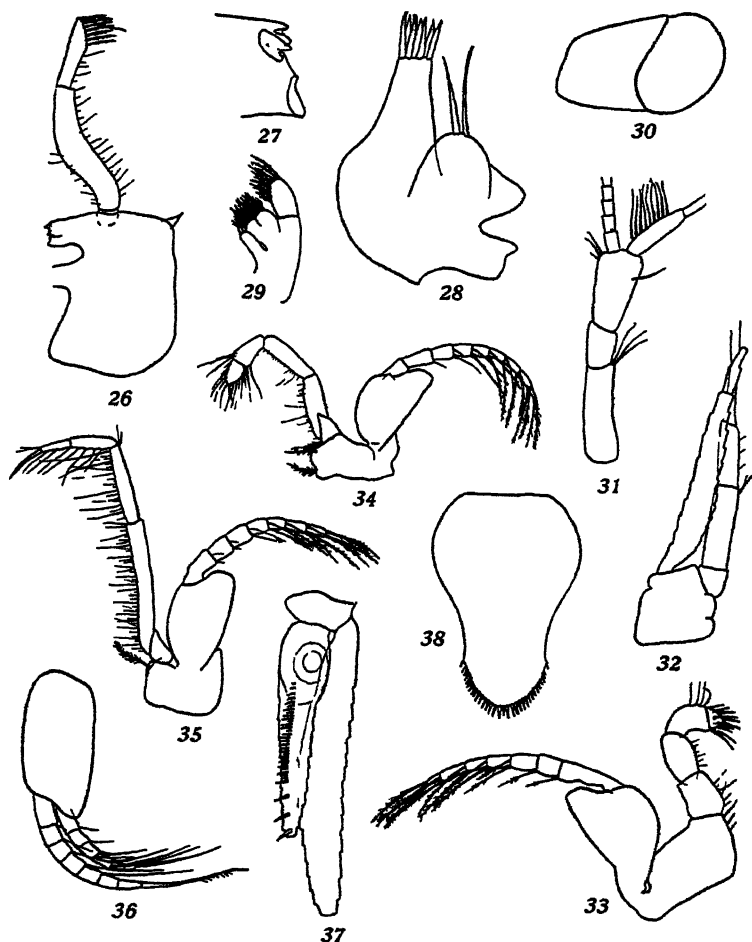
Figs. 26–38. *Mysidopsis elongata* S. J. Holmes

Fig. 26. Right mandible with palp. × 100.
Fig. 27. Cutting edge of the left mandible. × 330.
Fig. 28. First maxilla. × 330.
Fig. 29. Second maxilla. × 100.
Fig. 30. Eye. × 100.
Fig. 31. Peduncle of antennule. × 78.
Fig. 32. Antennal scale and peduncle. × 78.
Fig. 33. First thoracic limb. × 100.
Fig. 34. Second thoracic limb. × 78.
Fig. 35. Third thoracic limb. × 78.
Fig. 36. Fourth pleopod of the male. × 100.
Fig. 37. Uropods. × 78.
Fig. 38. Telson. × 100.

Length.—Adult specimens of both sexes, 6–7 mm.

Description.—Body slender; carapace very slightly produced in front into a short, wide-angled, bluntly pointed rostral plate which does not cover any part of the eyestalks.

Eyes large, cylindrical; cornea occupying rather less than half of the whole eye.

Antennular peduncle in the female rather long and slender, first joint longer than the third but shorter than the second and third combined. In the male the antennular peduncle is shorter and stouter than in the female and the setose lobe at the distal end is short and densely fringed with long setae.

Antennal scale about as long as the antennular peduncle, rather slender and narrow, setose all around, about seven times as long as broad; the distal joint equal to about one-fifth of the whole scale and marked off by a distinct articulation; antennal peduncle shorter than the scale, the terminal joint three-quarters the length of the preceding joint. In the male the antennal scale is somewhat shorter than in the female with the result that the antennal peduncle appears relatively longer, extending beyond the articulation of the distal joint of the scale.

Labrum forming a rather prominent conical papilla.

Mandibles with a distinct molar process; on the anterior outer angle of the body of the mandible, outside the articulation of the palp, is a prominent, sharp, forwardly directed spine. Maxillulae with a very short inner lobe armed with two long setae. Maxillae without exopod and without the setiferous expansion of the lobe from the second joint; palp two-jointed. Maxillipeds (first thoracic limbs) of the true *Mysidopsis* type, with the second and third joints of the endopod fused.

Third to the eighth thoracic limbs rather slender, the sixth joint divided into two by a transverse articulation; dactylus long and slender, longer than the distal division of the sixth joint. Basal plate of the exopod with the outer distal corner rounded and without spine.

Pleopods in the male of the type characteristic of the genus; fourth pair with the exopod rather longer than the endopod and terminating in a single long and rather stout plumose seta.

Telson short, two-third as long as the sixth abdominal somite, one and a half times as long as broad, linguiform in shape with the distal half much narrower than the proximal half; apex rounded and convex and armed with about thirty to thirty-four short spines; lateral margins terminating in a spine but otherwise unarmed.

Inner uropod one and a third times as long as the telson, inner margins armed with a row of spines from the statocyst to the apex, the spines arranged in series of smaller spines between larger ones in the proximal three-quarters of the row, distal three spines large, widely separated, and without smaller ones between them. Outer uropod twice as long as the telson.

Remarks.—Holmes's description and figures of *Mysidopsis elongata* are very imperfect but there is nothing in them that does not apply to the specimens I have described above. Zimmer's fuller and more complete description of *M. pacifica* allows a more certain comparison with these specimens. Zimmer's specimens differ from those here described as follows:

(1) in the form of the telson which has the apex much less convex and the spines terminating the lateral margins more sharply marked; (2) in the antennal peduncle which is longer than the first joint of the scale in the female, whereas

in my specimens it is shorter; (3) in having the terminal seta of the exopod of the fourth pleopod of the male relatively longer; (4) in not having the three separate distal spines on the inner margin of the inner uropod.

These differences are not great and for the moment I suggest, with some hesitation, that *M. elongata* and *M. pacifica* are synonymous. The two forms are very closely allied and agree in detail in the form of the mouth parts, particularly in the somewhat aberrant form of the maxillae, which lack an exopod, and the setiferous expansion of the lobe from the second joint. A further species, *M. munda,* described by Zimmer (1918) from Brazil, agrees with the present species in this respect. It is open to doubt whether *M. elongata* (including *M. pacifica*) and *M. munda* should not be placed in a new genus based on the character of the maxilla. *M. elongata* is easily distinguished from all the species of the genus by the form of the telson which, as Zimmer remarks, recalls that of *Macropsis slabberi* v. Ben.

Distribution.—M. elongata appears to be a common species at La Jolla. All the specimens were caught in tow-nettings taken at night and the species occurred regularly, if somewhat sparingly, throughout the two years during which the tow-nettings were taken. Holmes's specimens were caught at San Pedro, California, and *M. pacifica* at Callao, Chile.

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CONTRIBUTIONS TO A KNOWLEDGE OF THE MYSIDACEA OF CALIFORNIA

II. THE MYSIDACEA COLLECTED DURING THE SURVEY OF SAN FRANCISCO BAY BY THE U.S.S. "ALBATROSS" IN 1914

BY

W. M. TATTERSALL

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In this paper I record the results of my examination of the Mysidae which were collected during the survey of San Francisco Bay by the U.S.S. "Albatross" in 1914. I am greatly indebted to Professor C. A. Kofoid for kindly permitting me to undertake this work.

The collection was enormous from the point of view of the actual number of specimens contained, over 22,000 having been examined. They belong to five species of the genus *Neomysis*. Three of these, *N. mercedis, N. franciscorum,* and *N. costata* are not known elsewhere from the coast of California; one, *N. kadiakensis,* is also known from Alaska; and the fifth, *N. macropsis,* is new to science. I have redescribed and figured all the known species and have been able to clear up some doubtful points in their structure, as well as to elucidate more exactly than has been done heretofore the generic position of Holmes's (1900) species, *Mysis costata.*

As mentioned in my previous paper, this collection is entirely unlike the one from La Jolla; there is no species common to both. The San Francisco collection has a distinctly northern facies, all the species being closely allied to forms known from Alaska and the Bering Sea. Indeed it is probable that extended study of some of the northern forms may show that the Californian species are synonymous with them. *N. mercedis* is very similar to *N. intermedia, N. franciscorum* is difficult to separate from *N. rayii,* and *N. costata* is at least closely allied to *N. stelleri.* In the present state of knowledge, however, I have deemed it desirable to maintain the Californian forms as distinct species.

From a study of the physical conditions of San Francisco Bay described in the valuable and detailed report of Sumner and his col-

leagues (1914) it has been possible to show the effects of some of these conditions on the distribution of the species. It is clear, I think, that salinity is the most potent factor in restricting the occurrence and distribution of *N. mercedis* to the upper part of the Bay and, conversely but to a lesser degree, in confining *N. franciscorum* and *N. kadiakensis* to the middle and lower parts. In the case of *N. costata* a second factor, the nature of the sea floor, comes into play. This species is obviously a sand-dwelling form and its distribution in the Bay is therefore determined not only by salinity, but by the occurrence of suitable sandy ground. In the case of *N. macropsis,* the most abundant species in the collection, it was not possible to detect any obvious limiting factors.

SUBORDER MYSIDA

FAMILY MYSIDAE Dana

SUBFAMILY MYSINAE G. O. Sars

TRIBE MYSINI Hansen

Genus NEOMYSIS Czerniavsky
Genus HETEROMYSIS Czerniavsky

The numerous species now referred to this genus may be grouped together according to the following key:

Group I. Antennal scale long and narrow, terminating in an acute spiniform apex.

(a) Inner uropod with a dense row of spines on the lower surface near the statocyst.

  (i) Telson short, apex broadly truncate, spines on the lateral margin few and widely separated without smaller spines between them.

                N. awatchensis Brandt
                N. intermedia Czerniavsky
                N. mercedis Holmes

  (ii) Telson long and narrow, apex narrowly truncate, spines on the lateral margins numerous and rather widely separated without smaller spines between them.

                N. rayii Murdoch
                N. integer Leach
                N. franciscorum Holmes

  (iii) Telson long and narrow, apex narrowly truncate almost rounded, spines on the lateral margins numerous, rather crowded, without smaller spines between them.

                N. mirabilis Czerniavsky
                N. kadiakensis Ortmann
                N. japonica Nakazawa

(iv) Telson long and narrow, apex rounded, lateral spines numerous, crowded, with smaller spines between the larger ones.

> N. americana S. I. Smith
> N. spinosa Nakazawa
> N. czerniavskii Dershavin

(b) Inner uropod with only one or two spines on the lower margin near the statocyst.

(i) Telson long and narrow, apex narrowly truncate, lateral spines numerous without smaller spines between them.

> N. patagona Zimmer
> N. meridionalis Colosi
> N. monticellii Colosi

Group II. Antennal scale not unusually long, terminal joint rounded and not spiniform.

> N. longicornis M-Ed
> N. sagamiensis Nakazawa
> N. mitsukurii Nakazawa
> N. schrenckii Czerniavsky
> N. stelleri Dershavin
> N. costata Holmes
> N. dybowskii Dershavin
> N. indica Tattersall
> N. hodgartii Tattersall

Group II—those with the rounded apex to the antennal scale—have been referred to a separate genus, which has been named at various times *Acanthomysis* Czerniavsky, *Dasymysis* Holt and Beaumont, *Metamysis* Nakazawa, and *Orientomysis* Dershavin.

Zimmer (1915) in his revision of the genera of the Mysini regards all these genera as synonyms of *Neomysis*, mainly on the character of the pleopods, which are remarkably uniform throughout the species listed above.

The genus, however, is becoming somewhat unwieldy and includes, as listed above, twenty-four species. Some of these are probably not distinct and may, when material is available for study, be referred to the synonymy of other species. A large number of species will, however, still remain and it seems probable that Group II will have to be separated generically from the remainder on the character of the antennal scale. It forms a ready means of separating the species into two groups which may well be given generic rank. In such case the name *Acanthomysis* must be used to designate the second group of species.

In the material from San Francisco Bay submitted to me for examination, five species belonging to this genus in its widest sense

are represented, distributed according to the above grouping of species as follows:

I. a. i *N. mercedis* Holmes   II.   *N. costata* Holmes
I. a. ii *N. franciscorum* Holmes   *N. macropsis* n. sp.
I. a. iii *N. kadiakensis* Ortmann

In the three species belonging to the group I (*a*), which are present in this collection, I have observed two points which hitherto appear to have escaped notice.

The first of these is the presence of a small posterior setose lobe on the posterior pair of oostegites, projecting backward, and rather sharply marked off from the main oostegite. I suggest that this lobe functions as a bailer and keeps a current of water constantly flowing forward through the marsupial pouch from the posterior end.

The second point is the presence in the female of a rather long, delicate, somewhat curved and forwardly directed spiniform process on the median line of the last three thoracic sterna. It is somewhat difficult to suggest what purpose they serve. They may help in some way to support the oostegites and to divide the marsupial pouch into two chambers.

Neomysis mercedis Holmes
Figures 39–41

N. mercedis Holmes, 1897, p. 199, pl. 19, figs. 1–10.
N. mercedis Holmes, 1900, p. 223.

Locality.—An abundant species in the Bay; apparently most numerous in the upper bay, where it was taken on twenty-three occasions; it was found in the middle bay on four occasions and also in the lower bay.

Description.—Front margin of the carapace produced into a subquadrangular plate with rounded angles and with the front edge very slightly concave; plate broader than long; antero-lateral corners of the carapace long and acutely pointed.

Eyes, including stalk, about one and a half times as long as broad, cornea occupying slightly less than half of the entire eye in dorsal view.

Antennal scale long and narrow, about eight times as long as broad; terminal segment marked off by a distinct suture, about one-seventh of the scale in length and terminating in an acute spiniform apex; both inner and outer spines on the basal joint.

Thoracic limbs with the sixth joint of the endopod divided into from eight to ten subsidiary joints; basal plates of the endopod with spiniform outer distal angles.

Telson triangular in shape, about twice as long as broad at the base, narrowing to a truncate apex which is about one-sixth the width

of the base; apex armed with two pairs of spines—a large pair with one member at each angle of the apex and equal to one-twelfth the total length of the telson, and a smaller inner pair between the larger pair but only about one-quarter as long; lateral margins armed with from twelve to sixteen spines extending throughout the entire length of the margins; the proximal three or four spines are rather crowded
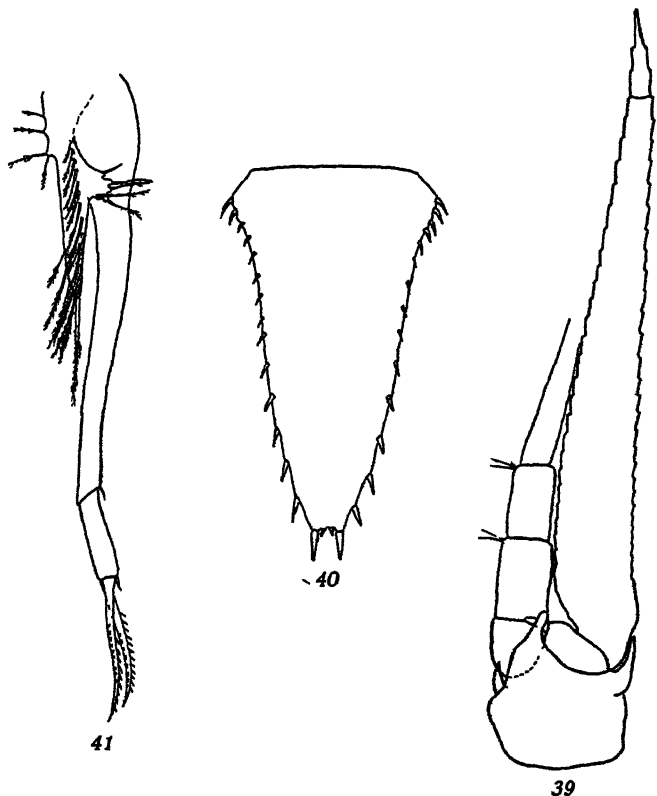
Figs. 39–41. *Neomysis mercedis*, S. J. Holmes.
Fig. 39. Antennal scale and peduncle.   × 78.
Fig. 40. Telson.   × 62.
Fig. 41. Fourth pleopod of the male.   × 78.

but the remaining spines are somewhat widely separated and increase in length distally.

Fourth pleopod of the male extending about halfway along the sixth abdominal somite; terminal joint of the outer branch less than one-quarter as long as the proximal and bearing distally two long, strong barbed spines, which are longer than the terminal joint.

Length.—Adult males and females, 15 mm.

Remarks.—This species belongs to the *Awatchensis* group of species within the genus. The group is distinguished by the acute spiniform apex of the antennal scale and the short broad triangular telson

with a truncate apex and relatively few, distantly placed spines arming the lateral margins without any smaller spinules in between.

Four species have been described which belong to this group, viz:

N. awatchensis Brandt   *N. nigra* Nakazawa
N. intermedia, Czerniavsky   *N. mercedis* Holmes

The first two of these species have been much confused in the literature, chiefly owing to the imperfect original description of *N. awatchensis* by Brandt and the fact that Czerniavsky described *N. intermedia* from an obviously immature specimen. In order to clear up the relationships and synonomy of these four species a brief account of their history is desirable.

The first species of the series to be described was *N. awatchensis* by Brandt (1851). The description is very short and inadequate. He did state, however, that his specimen was quite black.

Czerniavsky (1882), in the second part of his monograph on the group, redescribed and figured the species, apparently from Brandt's original specimens. In the same part of his monograph he also described and figured a new species which he called *Heteromysis intermedia*. He recognized, however, its close relationship to *M. awatchensis* but although he had numerous specimens at his disposal, his figures of the male pleopods were obviously made from an immature specimen.

In 1910 Nakazawa recorded *N. intermedia* from Japan without further description and described a new species *N. nigra,* also from Japan. He failed to recognize the relationship of *N. nigra* to *N. intermedia* and made no reference to Brandt's species at all. The specific name which he gave to his new species indicates, however, that it was black like *N. awatchensis.*

In 1921 I described *N. nigra* from China and *N. awatchensis* from Japan. I noted the resemblance of both species to *N. intermedia* but was misled by the description given by Czerniavsky of the male pleopods of the latter species and thereby failed to appreciate what I now think are the true relationships of the species. I did, however, point out the differences between what I believed to be *N. nigra* and *N. awatchensis.*

In 1923 Dershavin published a description of a species of *Neomysis* from Kamtschatka under the name of *N. awatchensis* Brandt, which agrees in close detail with the species I described under that name in 1921. He also confirms the differences between this form and *N. nigra* which I outlined in the same paper. Dershavin, however, is of the opinion that *N. awatchensis* Brandt and *N. intermedia* are synonymous.

Reviewing the whole literature, I now think that the *N. awatchensis* of Brand and of Czerniavsky is not the same as the species described under that name by Dershavin and myself, but is the same species as that described by Nakazawa and myself as *N. nigra.*

The species described by Dershavin and myself as *N. awatchensis* is the true *N. intermedia* of Czerniavsky.

The synonymy of these three species may therefore be set out as follows:

> *N. awatchensis* Brandt, 1851
>   = *Mysis awatchensis* Czerniavsky, 1882.
>   = *Neomysis nigra* Nakazawa, 1910
>   = *Neomysis nigra* Tattersall, 1921
> *N. intermedia,* Czerniavsky, 1882
>   = *Heteromysis intermedia* Czerniavsky, 1882
>   = *Neomysis intermedia* Nakazawa, 1910
>   = *Neomysis awatchensis* Tattersall, 1921
>   = *Neomysis awatchensis* Dershavin, 1923

It is now possible to indicate the relationships of *N. mercedis* to these two forms to which it is closely allied.

N. mercedis differs from *N. intermedia* in the following points:
(1) The antennal scale is relatively shorter and broader, eight times as long as broad, whereas in *N. intermedia* it is eleven times as long as broad.
(2) In the proportions of the joints of the outer branch of the fourth pleopod of the male. In *N. mercedis* the terminal joint is less than one-quarter the length of the proximal joint and is shorter than the terminal spines. In *N. intermedia* the terminal joint is half as long as the proximal and is longer than the terminal spines. In all other particulars the two species agree very closely.

N. mercedis differs from *N. awatchensis* in the following points:
(1) Size. *N. awatchensis* is 10 mm. when adult and *N. mercedis* reaches 15 mm.;
(2) Color. *N. awatchensis* is black and *N. mercedis* is certainly not black;
(3) The rostral plate of *N. awatchensis* is pointed while in *N. mercedis* it is broadly quadrangular in shape;
(4) The sixth joint of the thoracic endopods in *N. awatchensis* has only from three to six subsidiary joints, while in *N. mercedis* it has from eight to 10.

It will be seen from this summary that *N. mercedis* is closely allied to *N. awatchensis* and to *N. intermedia,* but differs from both sufficiently to be regarded as a distinct species.

Distribution.—N. mercedis has hitherto been recorded only from Lake Merced, a fresh-water lake on the San Francisco Peninsula, California.

Neomysis franciscorum Holmes

Figures 42–44

N. franciscorum Holmes, 1900, p. 223.
N. franciscorum Hansen, 1913, p. 178, pl. 9, figs. 3*a*, *b*.
N. franciscana Schmitt, 1919, p. 7.

Locality.—The most abundant species of *Neomysis* in San Francisco Bay. Most abundant in the lower, but extending throughout the middle and upper bay. It was taken outside the Bay on one occasion.
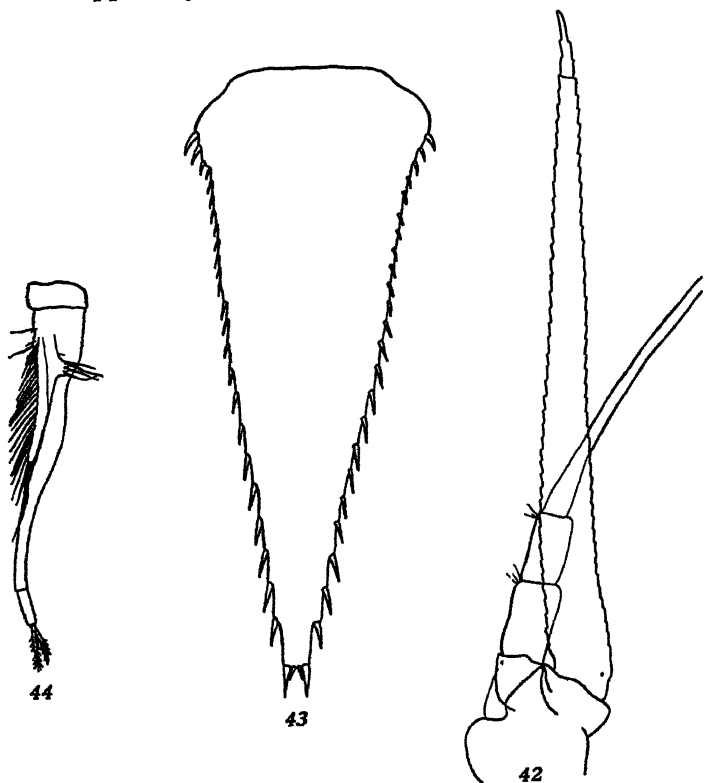
Figs. 42–44. *Neomysis franciscorum* S. J. Holmes.
Fig. 42.   Antennal scale and peduncle.   × 25.
Fig. 43.   Telson.   × 37.
Fig. 44.   Fourth pleopod of the male.   × 25.

Description.—Front margin of the carapace produced into a wide subquadrangular plate, less than half as long as broad, with rounded angles; the front margin of the plate depressed between the eyes so that it appears slightly concave in outline; as a matter of fact there is the slightest suspicion of a median spine.

Eye, including the stalk, one and a half times as long as broad, cornea occupying about one-third of the eye in dorsal view.

Antennal scale long and narrow, about twelve times as long as broad; terminal segment marked off by a distinct suture, about one-tenth of the scale in length and terminating in an acute spiniform apex; both inner and outer spines on the basal joint.

Thoracic limbs with the sixth joint of the endopods divided into from ten to fourteen subsidiary joints, basal plate of the exopods with an acute spiniform outer angle.

Telson long and narrowly triangular in shape; two and three-quarters times as long as broad at the base, rapidly narrowing to a slender but truncate apex which is about one-ninth the width of the base, apex armed with a long stout spine at each corner and a pair of smaller spines between, the small spines being less than half as long as the large spines; the lateral margins armed throughout their entire length with about twenty-five spines, increasing gradually in size distally, the distal spines more widely spaced than the proximal, the spines as a rule shorter than the distance between them; the apex of the telson between the last pair of spines on the lateral margins and the last pair of spines at the angles of the apex almost twice as long as broad and nearly twice as long as the last pair of lateral spines.

Fourth pleopod of the male extending to the distal end of the sixth abdominal somite; distal joint of the exopod only about one-seventh the length of the proximal joint and slightly shorter than the two terminal barbed spines.

Length.—Adult specimens of both sexes, 35 mm.

Remarks.—*N. franciscorum* belongs to that group of species of the genus characterized by the spiniform extremity of the antennal scale, by the long, somewhat narrowly triangular form of the telson with, however, the apex distinctly truncate, and by the fact that the spines arming the lateral margins of the telson have no small spines or spinules between them. Four described species are referable to this group, viz.:

N. integer (Leach)	*N. franciscorum* Holmes
N. rayii (Murdoch)	*N. toion* Dershavin

Of these four species, *N. toion* Dershavin, is undoubtedly synonymous with *N. rayii* Murdoch. No complete description of *N. rayii* has yet been published and Dershavin had perforce to rely on the very meager account of this species furnished by Murdoch. Dershavin recognized the close affinity of the two species. The most striking difference which he notes between the two is in the number of subsidiary joints in the sixth joint of the endopods of the thoracic limbs. Murdoch gives the number as from eight to nine in *N. rayii;* Dershavin found from 14 to 21 in *N. toion.* An examination of cotypes of *N. rayii,* which opportunity I owe to the kindness of the authorities of the United States National Museum, has shown that this difference does not exist in fact. There is no marked character separating the two forms and in my opinion they should be united.

Both Holmes and Hansen have compared *N. rayii* and *N. franciscorum*. Considerable doubt has existed as to whether the species were really distinct, though Hansen was inclined to regard them as separate and pointed out characters of the rostral plate and telson which appeared of sufficient magnitude to separate them. The examination of this large series of specimens from San Francisco Bay has convinced me that Hansen's opinion is correct. To the characters of the rostral plate and telson can be added the differences in the proportions of the eye and in the form of the fourth pleopod of the male.

The differences between *N. rayii* and *N. franciscorum* can best be expressed in tabular form as follows:

	N. rayii	*N. franciscorum*
Rostral Plate.—	More than half as long as broad	Less than half as long as broad
Eye.—	Nearly two and one-half times as long as broad; cornea one-third of the eye	Only twice as long as broad; cornea one-third of the eye
Telson.—	Terminal portion between last pair of spines on the lateral margin and the apex shorter than broad	Terminal portion between last pair of spines of the lateral margin and the apex longer than broad
Fourth Pleopod of the Male.—	Distal joint of the exopod one-third of the proximal	Distal joint of the exopod one-sixth of the proximal

N. franciscorum thus differs from *N. rayii* in the relatively shorter rostral plate, the shorter and stouter eye, the relatively shorter distal joint of the exopod of the fourth pleopod of the male, and the different form of the terminal portion of the telson.

Neomysis kadiakensis Ortmann
Figures 45 to 50

N. kadiakensis Ortmann, 1908, p. 8.
N. kadiakensis Schmitt, 1919, p. 7b, fig. 3e.

Locality.—Quite common in San Francisco Bay but not so abundant as *N. franciscorum*. Most often met with in the lower bay, less frequently in the middle bay, and on only five occasions in the upper bay.

Description.—Front margin of the carapace almost exactly as described above for *N. franciscorum*.

Eyes, including the stalk, about one and a half times as long as broad, cornea occupying two-fifths of the eye in dorsal view.

Antennal scale long and narrow, from thirteen to fourteen times as long as broad; terminal segment marked off by a distinct suture, about one-eighth of the entire scale in length and terminating in an acute spiniform apex; both inner and outer spines on the basal joint.

Thoracic limbs with the sixth joint of the endopod divided into from ten to twelve subsidiary joints; basal plate of the exopod with a spiniform outer distal angle.
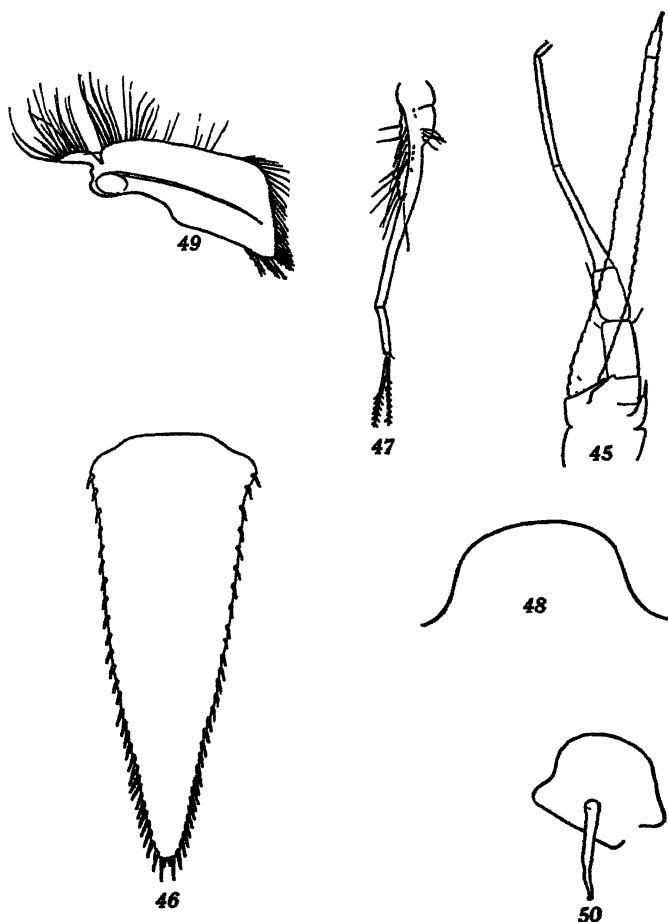
Figs. 45–50. *Neomysis kadiakensis* Ortmann.

Fig. 45. Antennal scale and peduncle. × 25.
Fig. 46. Telson. × 37.
Fig. 47. Fourth pleopod of the male. × 25.
Fig. 48. Rostral plate. × 78.
Fig. 49. One of the posterior oostegites to show the posterior lobe or "baler." × 78.
Fig. 50. Spiniform process on thoracic sternum. × 78.

Telson triangular in shape, about two and a half times as long as broad at the base, narrowing regularly to a slender truncate apex which is about one-tenth the width of the base; apex armed with two pairs of spines, the outer pair twice the length of the inner pair; lateral margins armed throughout their whole length by from twenty-

nine to thirty-five spines, the spines increasing in length toward the apex and becoming more crowded together; interval between the spines distally much less than the length of the spines.

Fourth pleopod of the male extending to the distal end of the sixth abdominal somite; terminal joint of the outer branch about one-quarter of the proximal joint and slightly shorter than the two long barbed spines at its apex.

Length.—Adult specimens, males and females, 20 mm.

Remarks.—This species is very closely allied to *N. franiscorum*. It is, however, a smaller species and differs clearly in the form of the telson. The latter in *N. kadiakensis* is proportionally more slender and tapering than in *N. franciscorum*. The apex is distinctly narrower and, though truncate, looks almost rounded. The spines arming the lateral margins are more numerous and distally much more crowded than in *N. franciscorum*. The two species also differ in the slightly different proportions of the joints of the exopod of the fourth pleopod of the male.

N. kadiakensis was described but not figured by Ortmann (1908) from specimens collected off the coast of Alaska. My specimens differ from Ortmann's description in the following points:

(1) The rostrum is described by Ortmann as bluntly triangular and similar to that of *N. vulgaris*. In my specimens it is true that there is the slightest suspicion of a median spine, but the rostral plate on the whole is quadrangular in outline with broadly rounded angles.

(2) The telson is described by Ortmann as having only from twenty to twenty-three spines on the distal two-thirds of the lateral margins, the proximal third being unarmed. I can only suppose that Ortmann overlooked the proximal spines which are frequently small and in dorsal view appear to be on top of the margin and not to project laterally. Otherwise Ortmann's description agrees with my specimens.

(3) The fourth pleopod of the male in Ortmann's specimens had the distal joint of the exopod half as long as the proximal and slightly longer than the terminal spines. This character is somewhat puzzling. I have seen specimens of *N. kadiakensis* taken off the California coast in somewhat deeper water than the present specimens, which agree with Ortmann's description in the proportions of the joints of the exopod of the fourth pleopod of the male. They are no larger than my largest males. I can only conclude that the San Francisco Bay specimens have not quite assumed the adult condition of the pleopods or that they represent a local shallow water variety.

The figure which Schmitt (1919) gives of the distal end of the telson of *N. kadiakensis* (taken, I presume, from the type specimen) agrees closely with my specimens here described.

N. kadiakensis is very closely allied to *N. mirabilis* (Czerniavsky) from northeastern Asia and differs only in the slightly different shape of the telson. In *N. mirabilis* the telson narrows much more toward the apex so that the spines arming the lateral margins look longer than in *N. kadiakensis*. It is doubtful if this difference is sufficiently great to be regarded as specific. Schmitt has described similar differences between young and fully grown *N. andersoni* = *N. czerniavskii*, Dershavin. *N. mirabilis* was described from specimens from 14 to 17.5 mm. in length and therefore smaller than adult specimens of *N. kadiakensis*. I have not observed these differences in small specimens of *N. kadiakensis* in the present collection. Otherwise the two species agree very closely in all their characters.

N. kadiakensis is also very closely allied to *N. japonica* Nakazawa but differs in the proportions of the two joints of the exopod of the fourth pleopod of the male. In *N. kadiakensis* the distal joint is one-quarter the size of the proximal, but in *N. japonica* it is only one-seventh of the proximal.

Neomysis costata (Holmes)

Figures 51–58

Mysis costata Holmes, 1900, p. 221, pl. 4, figs. 70–72.
Mysis costata Hansen, 1913, p. 177, pl. 9, figs. 2a–d.

Locality.—An abundant species in the middle bay; taken on three occasions only in the upper bay and once in the lower bay.

Description.—A small, compact, rather robust species; carapace produced in front into a short triangular rostral plate with a pointed apex; antero-lateral angles rounded.

Eyes short and stout with the stalks scarcely longer than the cornea.

Antennal scale extending beyond the distal end of the antennular peduncle by about one-quarter of its length; scale five times as long as broad, setose all round, with a small distal joint marked off by a distinct suture; a prominent spine on the outer corner of the joint from which the scale arises; antennal peduncle about half as long as the scale and shorter than the antennular peduncle.

Labrum produced into a prominent spiniform process.

First gnathopod with prominent lobes from the second and third joints.

Third to the eighth thoracic limbs rather stout; endopods with the carpus shorter than the merus; propodus divided into four or

five subjoints; dactylus well developed, long and acute; basal plate of the exopods with one, sometimes two, small spines on the outer distal corner.

Figs. 51–55. *Neomysis costata* (S. J. Holmes).

Fig. 51.   Labrum.   × 78.
Fig. 52.   First thoracic limb.   × 100.
Fig. 53.   Second thoracic limb.   × 100.
Fig. 54.   Antennal scale and peduncle.   × 100.
Fig. 55.   Fourth pleopod of the male.   × 100.

Somites of the abdomen with transverse ridges which Hansen has interpreted as dorsal foldings of the skin.   There are three on the first somite and two on the second to the fifth somites.   The sixth somite has the first transverse ridge produced in the mid-dorsal line into a small triangular plate.   Behind the ridge the somite is sculptured and the posterior border of the somite is also slightly acuminate in

the mid-dorsal line.   The sixth somite is one-fifth longer than the fifth.

Telson nearly twice as long as the sixth abdominal somite and two and a half times as long as broad at the base: lateral margins tapering to a narrow but distinctly truncate apex; lateral margins armed throughout their entire length with numerous large spines which
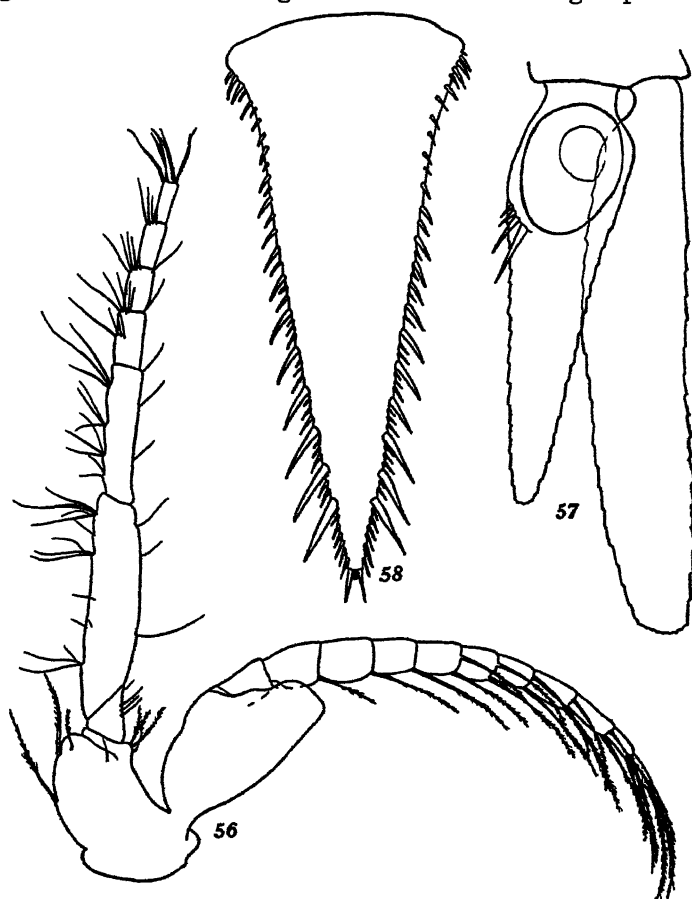
Figs. 56–58. *Neomysis costata* (S. J. Holmes).
Fig. 56.   Third thoracic limb.   × 100.
Fig. 57.   Uropods.   × 100.
Fig. 58.   Telson.   × 100.

increase considerably in length toward the apex; on the distal part of the margins the spaces between the larger spines are occupied by smaller spines, two, three, or four in each space; apex narrowly truncate and armed with four spines, the outer spines more than twice as long as the inner.

Inner uropod shorter than the telson, with a row of four or five strong spines on the lower inner face in the region of the statocyst. Outer uropod slightly longer than the telson.

Pleopods of the male of the typical *Neomysis* form; fourth pair modified, rather stout, outer branch about twice as long as the inner branch, two-jointed, terminal joint one-fifth the length of the proximal joint and armed with two long stout plumose setae three times as long as the terminal joint.

Length.—Adult specimens, in both sexes, 8 mm.

Remarks.—This is a very distinct species of the genus. Hansen had only a single female at his disposal so that, while realizing that the species could not be retained in the genus *Mysis,* he was unable to refer it to its appropriate genus. He expressed the opinion, however, that it approached most nearly to the genus *Neomysis.* Examination of the pleopods of the male confirms Hansen's suspicions. As that genus is defined by Zimmer (1913) *N. costata* must clearly be included in it. It belongs, however, to that section of the genus characterized by the short antennal scale with the rounded apex and is most nearly related to that group of species which have been included in the genus *Orientomysis* Dershavin = *Metamysis* Nakazawa (*nec* Sars). *N. costata* is most nearly allied to *N. stelleri* Dershavin. Both species have the transverse ridges on the abdominal somites and both have telsons of similar appearance and armature. In these two features they stand sharply marked off from all other species of the genus. Whereas *N. stelleri* is a large species, measuring 19 mm. in length and having the antennal scale seven times as long as broad, *N. costata* is a small form, measuring only 8 mm. in length and having the antennal scale five times as long as broad.

Neomysis macropsis, sp. nov.

Figures 59–65

Locality.—A very abundant species all over the bay, occurring equally in upper, middle and lower parts. It was taken on one occasion outside the bay at Station 5219.

Description.—A very distinctive species of the genus resembling species of the genus *Macropsis* in general external form; body slender, cephalo-thorax much narrower in front than behind; carapace leaving the last two thoracic somites exposed; rostral plate very slightly produced and broadly and evenly rounded, antero-lateral corners produced into long acute spines.

Eyes greatly elongated and rather narrow; whole eye (including eyestalk) three and one-third times as long as broad; eyestalk three-quarters of the entire length of the eye; visual portion of the eye one-quarter of the entire length of the eye and wider than long; eye longer than the antennal scale and the antennal peduncle and extending as far forward as the distal end of the second joint of the

antennular peduncle; the whole eye is remarkably like that of the genus *Macropsis*.

Antennular peduncle in the female long and slender, about one-seventh longer than the eye; basal joint almost as long as the two
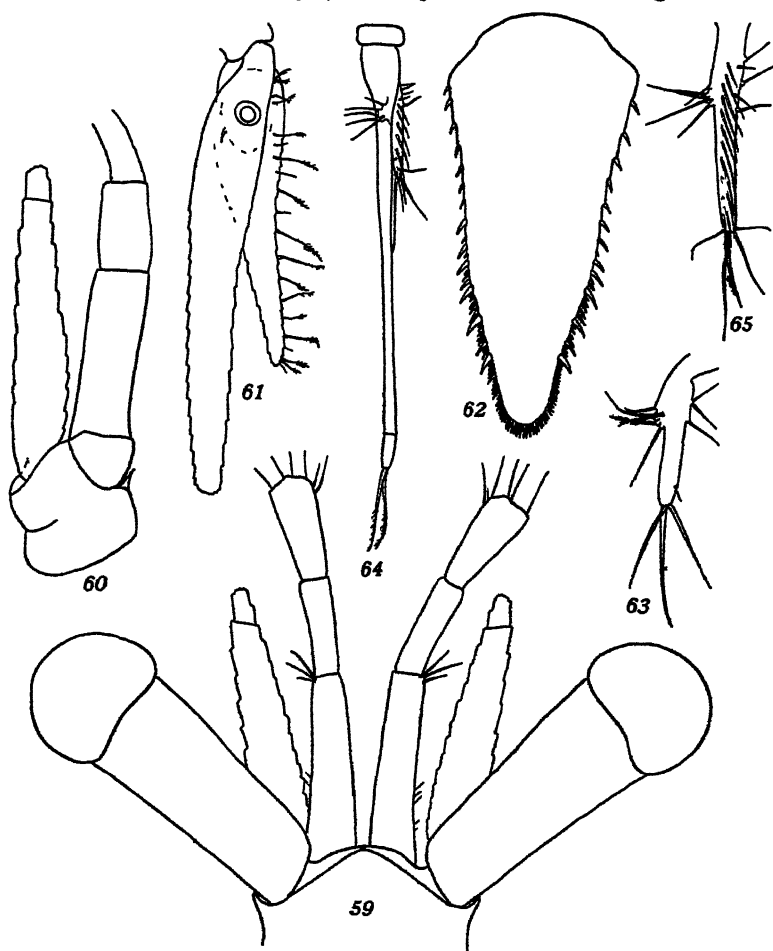
Figs. 59–65. *Neomysis macropsis* sp. nov.

Fig. 59. Anterior end of a female, to show rostral plate, eye, antennule
Fig. 60. Antennal scale and peduncle. × 78.
Fig. 61. Uropods. × 60.
Fig. 62. Telson. × 78.
Fig. 63. Third pleopod of the male. × 78.
Fig. 64. Fourth pleopod. × 62.
Fig. 65. Fifth pleopod of the male. × 78.

distal joints combined; second and third joints subequal; in the male the antennular peduncle is somewhat shorter and a little stouter than in the female and carries a well developed lobe armed with a brush of long fine setae.

Antennal scale much shorter than the antennular peduncle, extending as far forward as the eye and the listal end of the second joint of the antennular peduncle; six times as long as broad, with a well marked suture dividing off a distal portion equal to about one-ninth of the whole scale; scale narrowly lanceolate in shape, apex rounded and not produced into a spine, margins with setae all round; basal joint, from which the scale arises, with a prominent spine on both inner and outer corners.

Antennal peduncle shorter than the scale, with the second joint nearly twice as long as the third.

Mouth parts showing no very marked divergence from those of the genus *Neomysis* except that there is no spinous process from the labrum; the latter is raised into a conical protuberance which, however, is bluntly rounded and not acute.

First gnathopod (first thoracic limb) with a well developed lobe on the inner margin of the second joint; third and fourth joints somewhat expanded, but hardly lobed.

Third to eighth thoracic limbs long and slender, the meral and carpal joints of the endopod especially appear to be longer and more slender than is usual in this genus; propodal joint divided into from five to seven subjoints, terminal joint (dactylus) very small and reduced; the endopod terminated by two or three long simple setae and a single stouter barbed seta amid which the smaller dactylus lies hidden; basal plate of the exopods with the outer distal corner rounded.

Sixth abdominal somite longer than any of the anterior somites, about one-seventh longer than the fifth somite and one and a half to twice as long as the remaining somites.

Telson as long as the sixth somite of the abdomen, linguiform in shape, about twice as long as broad at the base, margins narrowing to a bluntly rounded apex; the armature of the lateral margins is very distinctive; there are about thirteen larger spines somewhat widely spaced and extending about five-sixths of the length of the margins from the base; between the last four or five larger spines there are a varying number of smaller spines, but between the more proximal there are no smaller spines; the apex of the telson between the last large spines is armed by a very closely set row of small spines which are more like the closely set teeth of a saw than a series of articulated spines.

Inner uropod about one-sixth longer than the telson, with a single spine on the inner lower face near the statocyst; along the outer margin, in addition to the usual long marginal setae, there are a number of small delicate plumose setae. Outer uropod one and a half times as long as the telson.

Fourth pleopod of the male extending back to the posterior end of the sixth abdominal somite; exopod greatly elongate and very slender, two-jointed; proximal joint about ten times as long as the distal joint, the latter terminating in two stout plumose setae, more than twice as long as the terminal joint.

Length.—Adult specimens of both sexes, from 12 to 14 mm.

Remarks.—This is a very aberrant species of the genus *Neomysis*. It differs from other species of the genus in the short bluntly rounded antennal scale, in the absence of a spinous process on the labrum, in the long cylindrical form of the eyes, in the form of the rostral plate, and in the long and slender thoracic limbs with their reduced dactyli. The armature of the telson is reminiscent to a certain extent of the group of species of which *N. longicornis* is the type, but the peculiar arrangement of the apical spines is quite distinctive. In general appearance the species recalls very strongly the genus *Macropsis* and but for the quite simple third pleopod of the male and the shape and length of the telson, it could quite well be referred to that genus. In fact it can be regarded as a *Macropsis* in which the reduction of the third pleopod of the male to the female condition had proceeded to completion.

DISTRIBUTION OF THE SPECIES OF MYSIDACEA COLLECTED DURING THE BIOLOGICAL SURVEY OF THE SAN FRANCISCO BAY

Geographical—

Our knowledge of the Mysidacean fauna of the west coast of America is at present too fragmentary to permit any general conclusions as to the distribution of any of the species or as to the bearing of the evidence of distribution on the unity or otherwise of the faunal area between the Aleutian Islands and California. I am at present engaged in studying a large collection of Mysidacea received from the United States National Museum. When that task is completed it may be possible to deal more fully with this problem. In the meantime it is only possible to state that of the five species here recorded from San Francisco Bay, only one, *N. kadiakensis,* has been recorded from outside that area. It is known from the Kadiak Islands off the coast of Alaska.

Distribution in San Francisco Bay—

(For lists of the species taken at the dredging and hydrographic stations and also in the shore collections during the survey, see pp. 337–341.)

Mysidacea were mainly collected in tow-nettings at the 313 hydrographic stations inside the Bay. Mysids were taken in the plankton at 163 of these stations, i.e., 52 per cent, and were collected at only 12 dredging stations out of 133, or 9 per cent. In addition they were captured during shore collecting operations on three occasions.

These facts demonstrate the essentially free-swimming habits of these animals.

Table 1 is a record of the distribution of mysids at the tow-netting and dredging stations at which they were captured. It is designed to show the divisions of the Bay in which the hauls were made and the species they contained.

Table 2 gives the actual numbers of specimens of each species caught in each of the six periods of the survey.

Table 3 presents an analysis of the occurrence of four species in relation to the salinity of the water at the time of capture.

Tables 4–8 give analyses in detail of the occurrence of each species so as to show the number of hauls in which it occurred in each division of the Bay and at each period of the survey.

Table 1—

This table brings out the fact that mysids were most abundant, on the whole, in the middle and lower portions of the Bay. It further makes it clear that *Neomysis mercedis* is preponderantly an upper bay species and that *N. costata* is equally a middle bay species. *N. macropsis* occurs uniformly in all three divisions of the Bay and is the most abundant mysid in the area. *N. kadiakensis* is confined practically to the middle and lower divisions, rarely occurring in the upper bay. *N. franciscorum* has a distribution in the Bay closely similar to that of *N. kadiakensis* but ranging somewhat more frequently into the upper bay. Only two species, *N. franciscorum* and *N. macropsis*, were found outside the Bay.

Table 2—

This table shows mysids were generally less abundant during the summer months than during those of the winter. I am unable to suggest any explanation for this unless it is that the low summer temperatures (Sumner, *et al.*, 1914) delay the reproductive period and the growth of the young to such an extent that the full effects of the breeding season on the mysid population are not apparent until late in the autumn. At the onset of the breeding season the population of mysids for the year would be at its minimum and if the breeding season is late, because of a low summer temperature, that minimum population may continue until well into the autumn. *Neomysis macropsis* shows this phenomenon clearly and it applies with equal clearness to the total mysids captured. The remaining species are not so markedly less abundant in the summer months as *N. macropsis*, but

all show a distinct fall in numbers at the fourth period of the year, early in October.

Table 3—

   This table, I think, gives a clue to one factor which appears to determine the distribution of species of mysids in the Bay, namely, the salinity of the water. It shows clearly the marked preference of *N. kadiakensis, N. franciscorum,* and *N. costata* for higher salinities and of *N. mercedis* for lower.

   N. mercedis apears to be a species which is adapted to sea water of low salinity and its distribution in San Francisco Bay, where it is practically confined to the upper bay, coincides with a region of low salinity.

   N. kadiakensis, N. franciscorum, and *N. costata,* which are apparently more marine in their habits and less specialized than *N. mercedis* in the matter of salinity, are more abundant in the lower and middle portions of the Bay where the salinity is higher, and they rarely penetrate into the upper bay.

   N. macropsis is not included in this table as it appears to be unaffected by variations in salinity but occurs more or less uniformly throughout the whole Bay.

Table 4—

   Neomysis franciscorum is found in all divisions of the Bay but is markedly scarcer in the upper bay than in the other divisions.

Table 5—

   Neomysis kadiakensis. This species is found regularly in the middle and lower bays, but it is much less frequently caught in the upper bay. Its absence from the latter, except during one period of the survey, is very striking and as salinity is the one factor in which the upper bay is most sharply distinguished from the rest of the Bay, it would appear that *N. kadiakensis* is much less tolerant of changes in salinity than *N. franciscorum.*

Table 6—

   Neomysis mercedis is very definitely an upper bay species, salinity being the determining factor which limits its distribution and confines it to this restricted area.

Table 7—

Neomysis macropsis is the most abundant and evenly distributed species of *Neomysis* in the Bay. It does not appear to be affected by either temperature or salinity.

Table 8—

Neomysis costata is practically confined to the middle area of the Bay. Salinity is at least one factor limiting its distribution (see table 3), and probably also accounts for its absence from the upper bay. Another factor, however, is operating which limits its distribution to the middle bay, for the salinity of the water in the lower bay is not sufficiently different from that of the middle bay to preclude its living there. This second factor appears to be the character of the bottom. A glance at the map illustrating the charactering of the bottom of San Francisco Bay will show that the bottom of the middle bay is more sandy and less muddy than in either of the other two regions (see Sumner *et al.*, 1914, pl. 5). It is firmer, harder, and cleaner, probably kept so by the scouring action of the water entering and leaving the Bay at the Golden Gate. *N. costata* apparently prefers a bottom of this character and avoids the muddier parts of the Bay. For this species, at any rate, there is clear evidence of two factors limiting its distribution, the salinity of the water and the character of the bottom.

SPECIES OF *Neomysis* TAKEN AT ALBATROSS DREDGING STATIONS
DURING THE YEARS 1912 AND 1913

Station number	Date	Depth in fathoms	Character of bottom	Neomysis franciscorum	Neomysis kadiakensis	Neomysis mercedis	Neomysis macropsis
Middle D. 5712	2/16/12	10½–14¾	Course gray sand and shell fragments				2
Outside D. 5735	3/11/12	9¾–10	Fine dark, very clean sand				2
Upper D. 5761	4/ 2/12	3 – 5	Soft muddy sand or sandy mud and vegetable debris			1	
Middle D. 5764	4/ 3/12	4¾– 2¼	Gelgrass	1			
Middle D. 5777	4/17/12	3½– 2¾	Large rounded weedy rocks		1		
Middle D. 5778	4/17/12	3½– 2¼	Fine clean, gray sand and medium sized rounded stones		1		
Middle D. 5797	10/29/12	8½– 7½	Sand		2		
Middle D. 5798	10/29/12	8 – 7½	Mud	5	16		
Lower D. 5803	10/30/12	9½– 8	Soft mud	5			
Lower D. 5805	10/30/12	5¼– 5	Soft mud and worm tubes	2			
Middle D. 5822	12/17/12	5 – 6	Smooth mud	1			
Middle D. 5828	1/20/13	16½–10¼	Soft tenacious mud		12		
Middle D. 5830	1/21/13	11 – 8½	Fine uniform dark gray sand to tenacious mud		2		

SHORE COLLECTIONS OF *Neomysis*

1912

February 2: Sausalito
  Neomysis macropsis, 2
April 22: Red Rock
  Neomysis franciscorum, 1
  Neomysis mercedis, 1
  Neomysis macropsis, 200

1913

March 1: Sausalito
  Neomysis macropsis, 1
  Neomysis costata, 10

SPECIES *Neomysis* TAKEN AT ALBATROSS HYDROGRAPHIC STATIONS
DURING THE YEARS 1912 AND 1913

Station number	Date	Neomysis franciscorum	Neomysis kadiakensis	Neomysis mercedis	Neomysis macropsis	Neomysis costata	Temperature	Salinity
Upper bay 4987	2/20/12	7		28	35		12.4	15.36
Upper bay 4988	2/20/12				2		12.75	15.46
Upper bay 4989	2/20/12			7	10		12.8	21.27
Upper bay 4990	2/20/12	1			76		12.7	24.20
Upper bay 4991	2/20/12	18		1	287		12.7	24.40
Upper bay 4992	2/21/12	1			4		12.45	21.45
Middle bay 4993	2/21/12	2			5		12.5	23.42
Middle bay 4994	2/21/12	14			21		12.5	24.56
Middle bay 4995	2/21/12	1			11		12.45	26.40
Middle bay 4996	2/21/12	19			9		12.7	27.96
Middle bay 4997	2/21/12				17		12.6	28.74
Middle bay 4998	2/21/12	30	12		15	5	12.35	28.66
Middle bay 4999	2/21/12	19			37	25	12.35	28.19
Middle bay 5000	2/21/12	2			1	9	12.2	31.09
Lower bay 5001	2/23/12				3		12.35	27.91
Lower bay 5003	2/23/12	88			90		12.65	28.31
Lower bay 5004	2/23/12	12			190		12.65	28.08
Lower bay 5005	2/23/12	2			500		12.65	27.85
Lower bay 5007	2/23/12	38	1		56		12.3	27.06
Lower bay 5008	2/23/12	12			9		12.3	27.76
Lower bay 5009	2/27/12	13			21		11.1	28.10
Lower bay 5010	2/27/12				98		11.5	27.36
Lower bay 5011	2/27/12	2			1100		10.75	27.26
Lower bay 5012	2/27/12	47	1		422		11.7	27.73
Lower bay 5013	2/27/12	66			34		11.7	27.77
Lower bay 5014	2/27/12	3			3		11.6	28.11
Lower bay 5015	2/27/12	1			9		11.65	28.26
Lower bay 5016	2/27/12	2			44		11.6	28.26
Upper bay 5082	4/23/12	7			534		12.7	18.25
Upper bay 5084	4/23/12	1		6	168		13.1	13.13
Upper bay 5085	4/23/12			22	65		13.35	8.09
Lower bay 5089	4/26/12	2			76		12.25	27.45
Lower bay 5090	4/26/12	5		*	206		12.3	27.17
Lower bay 5091	4/26/12	21	1		53		12.95	26.30
Lower bay 5092	4/26/12	28			55		13.05	24.85
Lower bay 5093	4/26/12	8			6		13.5	27.01
Lower bay 5094	4/26/12	3			3		14.05	27.12
Lower bay 5095	4/26/12	8			15		13.95	27.17
Middle bay 5097	4/29/12	10	5		20		11.05	31.48
Middle bay 5098	4/29/12	17			67	18	11.4	30.59
Middle bay 5099	4/29/12	4			20	37	11.55	30.37
Middle bay 5100	4/29/12	2			6		11.95	29.16

* Fragments

SPECIES *Neomysis* TAKEN AT ALBATROSS HYDROGRAPHIC STATIONS
DURING THE YEARS 1912 AND 1913

Station number	Date	Neomysis franciscorum	Neomysis kadiakensis	Neomysis mercedis	Neomysis macropsis	Neomysis costata	Temperature	Salinity
Middle bay 5101	4/29/12	58	2		35	75	11.95	28.30
Middle bay 5104	4/29/12	6	14		76		12.95	22.96
Upper bay 5105	4/30.12				*		12.9	21.52
Upper bay 5106	4/30/12	5		4	250		12.95	21.00
Upper bay 5107	4/30/12	32		1	822		13.05	20.42
Upper bay 5110	4/30/12			80	170		13.75	13.37
Lower bay 5113	5/ 1/12	25		1	5		12.7	27.59
Lower bay 5114	5/ 1/12	17			20		13.05	27.52
Lower bay 5115	5/ 1/12	40	1		29		13.25	27.01
Lower bay 5118	5/ 1/12	2			10		14.25	26.94
Middle bay 5120	5/ 6/12	10	5		9	1	13.2	27.17
Middle bay 5122	5/ 6/12	19	10		36	9	13.45	24.85
Middle bay 5123	5/ 6/12	52	1		12		13.7	24.29
Middle bay 5125	5/ 6/12	12	6		25		13.75	24.40
Middle bay 5126	5/ 6/12	30	16		82		13.55	25.20
Middle bay 5127	5/ 6/12	10	16		93		13.75	23.60
Middle bay 5128	5/14/12	18	1		135	4	12.5	28.35
Middle bay 5130	7/22/12				3		14.3	31.20
Middle bay 5131	7/22/12				1	4	15.15	30.28
Middle bay 5133	7/22/12				342		15.9	29.26
Middle bay 5136	7/22/12	12		1	16		16.9	28.00
Middle bay 5137	7/22/12	60	149	1	28	2	16.8	27.02
Lower bay 5138	7/23/12	35	60		32	5	16.0	29.25
Lower bay 5140	7/23/12	4	200		40	20	17.05	28.50
Lower bay 5141	7/23/12				1		17.7	28.10
Lower bay 5143	7/23/12				45		18.35	27.65
Lower bay 5144	7/23/12	5			81		18.4	27.71
Upper bay 5146	7/24/12	7			40	2	17.0	27.16
Upper bay 5147	7/24/12	4		1		2	17.6	25.81
Upper bay 5149	7/24/12	3		62	122		19.1	16.90
Middle bay 5150	7/29/12		13				17.65	24.40
Middle bay 5151	7/29/12	3		1	25		17.4	26.16
Middle bay 5152	7/29/12				20		17.1	26.39
Middle bay 5154	7/29/12	1			150		16.2	28.43
Middle bay 5155	7/29/12	6	3		12		15.35	29.72
Middle bay 5156	7/29/12	3			26		14.5	30.97
Lower bay 5159	7/30/12	3			15		19.25	27.86
Lower bay 5160	7130/12	1			8		18.75	27.90
Lower bay 5161	7/30/12	14			4		17.75	28.33
Lower bay 5164	7/30/12	32			35		16.45	29.14
Lower bay 5165	7/30/12	8			5		15.40	30.47
Upper bay 5166	7/31 12			38	33		18.5	11.42

* Fragments

SPECIES *Neomysis* TAKEN AT ALBATROSS HYDROGRAPHIC STATIONS
DURING THE YEARS 1912 AND 1913

Station number	Date	Neomysis franciscorum	Neomysis kadiakensis	Neomysis mercedis	Neomysis macropsis	Neomysis costata	Temperature	Salinity
Upper bay 5168	7/31/12	8		57	150		18.65	16.78
Upper bay 5172	7/31/12	8		1	58	2	16.45	28.74
Upper bay 5176	7/10/12	28		1	132		16.65	24.11
Upper bay 5177	7/10/12	2		8	37		16.70	21.91
Lower bay 5180	10/ 8/12	1			8		14.0	32.60
Lower bay 5183	10/ 8/12				14		16.3	31.31
Lower bay 5184	10/ 8/12	27		1	300		17.2	31.28
Lower bay 5186	10/ 8/12	3			385		17.1	31.30
Middle bay 5188	10/ 9/12	6	3		6	10	13.45	32.14
Middle bay 5190	10/ 9/12	3			6	1	14.05	32.70
Middle bay 5193	10/ 9/12	40	14		26		14 6	32.08
Upper bay 5196	10/10/12			1	112		16.2	16.67
Upper bay 5198	10/10/12	7			184		16.05	21.17
Upper bay 5199	10/10/12	7			137		16.0	24.96
Middle bay 5211	10/11/12	1			20		15.35	28.97
Middle bay 5216	10/12/12	6	3		3	1	16.50	32.13
Outside 5219	10/15/12	4			12		10.90	33.98
Middle bay 5228	11/ 4/12	5	11		6	9		
Lower bay 5232	11/11/12				40			
Upper bay 5242	11/26/12		5	1	459		11.95	17.98
Upper bay 5244	11/26/12				177		12.4	24.22
Upper bay 5245	11/26/12				89		12.35	27.45
Upper bay 5246	11/26/12				18		12.4	27.06
Upper bay 5247	11/26/12				7		12.2	28.53
Lower bay 5248	11/27/12				26		12.95	29.85
Lower bay 5249	11/27/12	1			38		12.95	29.33
Lower bay 5254	11/27/12	7	40		18		12.35	29.93
Lower bay 5255	11/27/12	16	94		12		12.40	30.58
Lower bay 5257	11/27/12				429			
Lower bay 5261	11/27/12				691			
Upper bay 5264	12/ 3/12		10		35		11.6	25.18
Upper bay 5266	12/ 3/12	20	15	100	534		11.5	19.80
Upper bay 5267	12/ 3/12	12	2	388	340		11.05	14.02
Lower bay 5273	12/ 3/12	5	1		884		12.05	29.95
Lower bay 5275	12/ 3/12	55	2				12.05	30.24
Lower bay 5276	12/ 3/12	39	2		6		11.95	30.43
Middle bay 5277	12/ 3/12				12		11.05	32.32
Middle bay 5282	12/ 3/12				5		11.45	30.93
Middle bay 5283	12/ 3/12				27		11.4	29.37
Middle bay 5284	12/ 3/12				28		11.25	28.18
Upper bay 5286	1/13/13				560		7.15	23.99
Upper bay 5287	1/13/13				594		7.05	22.79

SPECIES *Neomysis* TAKEN AT ALBATROSS HYDROGRAPHIC STATIONS
DURING THE YEARS 1912 AND 1913

Station number	Date	Neomysis franciscorum	Neomysis kadiakensis	Neomysis mercedis	Neomysis mactopsis	Neomysis costata	Temperature	Salinity
Upper bay 5288	1/13/13			6			6.9	20.66
Upper bay 5291	1/13/13			5	184		6.1	14.74
Upper bay 5292	1/13/13			7			6.1	15.03
Middle bay 5298	1/20/13	93	150		353	72	8.85	29.90
Middle bay 5399	1/20/13	8	14		18	5	8.75	29.03
Middle bay 5300	1/20/13				50	2	8.65	27.01
Middle bay 5301	1/20/13	4	5				8.35	26.55
Middle bay 5302	1/20/13	9	3	1	264		8.25	24.80
Middle bay 5303	1/20/13	2	1				8.1	20.20
Middle bay 5304	1/20/13	1	2	1			8.0	20.35
Lower bay 5305	1/20/13	2	17				8.7	29.30
Lower bay 5306	1/20/13		8		230		8.75	28.76
Lower bay 5307	1/20/13	1	2				8.25	27.87
Lower bay 5308	1/21/13	11			1		8.15	27.86
Lower bay 5310	1/21/13	170	34		750		7.7	28.48
Lower bay 5312	1/21/13	27	1		461		7.65	28.77
Lower bay 5313	1/27/13	13	5				8.1	28.70
Lower bay 5314	1/27/13	40	6				8.3	28.41
Lower bay 5316	1/27/13	44	5		726		8.6	27.36
Lower bay 5317	1/27/13	19			193		8.6	26.81
Lower bay 5319	1/27/13	47	6		430		8.75	26.41
Lower bay 5320	1/27/13	2			3		9.0	27.36
Middle bay 5321	1/27/13	1			129		8.45	18.41
Middle bay 5322	1/27/13	1			397		8.45	19.10
Middle bay 5323	1/28/13				166		8.8	22.56
Middle bay 5324	1/28/13				20		8.9	26.49
Middle bay 5325	1/28/13				74		9.05	23.88
Middle bay 5326	1/28/13				145		9.15	27.12
Middle bay 5328	1/28/13				2		9.35	29.48
Upper bay 5330	7/21/13				31		16.5	26.44
Middle bay 5336	7/21/13				40		15.1	30.39
Middle bay 5337	7/21/13	6			20		16.95	29.12
Middle bay 5338	7/21/13	4			8		15.45	28.51
Middle bay 5339	7/21/13	1					15.95	28.23
Middle bay 5341	7/21/13	17			92		15.45	29.21
Lower bay 5343	7/21/13				1		17.4	28.73
Lower bay 5346	7/21/13	3					20.6	27.83
Lower bay 5347	7/21/13	2					16.4	29.32

TABLE 1

NUMBERS OF HAULS IN WHICH MYSIDS OCCURRED IN DIFFERENT DIVISIONS OF
SAN FRANCISCO BAY

Divisions of the bay	Total number of hauls in which mysids occurred	Neomysis francis-corum	Neomysis kadiakensis	Neomysis mercedis	Neomysis macropsis	Neomysis costata
TOW-NETTING—						
Upper.............. ..	38	19	4	22	35	3
Middle........	60	44	24	5	56	18
Lower..	65	53	20	3	57	2
Outside....	1	1			1	
DREDGING—						
Upper.....	1			1		
Middle....	9	3	6		1	
Lower..	2	2				
Outside...	1				1	

TABLE 2

ACTUAL NUMBERS OF THE VARIOUS SPECIES OF MYSIDS CAUGHT IN SAN FRANCISCO
BAY DURING EACH PERIOD OF THE SURVEY

Period	Neomysis francis-corum	Neomysis kadiakensis	Neomysis mercedis	Neomysis macropsis	Neomysis costata	Total mysids
I. 2/13/12– 2/27/12	400	14	36	3029	39	3518
II. 4/23/12– 5/ 6/12	452	78	114	3123	144	3911
III. 7/22/12– 7/31/12	217	425	162	1292	37	2133
IV. 10/ 7/12–10/12/12	136	31	11	1416	21	1609
V. 11/25/12–12/ 5/12	155	171	489	3835		4650
VI. 1/13/13– 1/28/13	495	259	20	5750	79	6603

TABLE 3

OCCURRENCE IN RELATION TO SALINITY

Salinity	N. kadiakensis	N. franciscorum	N. mercedis	N. costata
32+	20	56	0	12
31+	5	42	1	9
30+	98	142	0	59
29+	285	216	0	82
28+	269	539	2	131
27+	164	441	2	5
26+	12	97	1	2
25+	26	34	1	2
24+	33	188	2	9
23+	16	12	0	0
22+	14	6	0	0
21+	0	15	19	0
20+	3	35	8	0
19+	15	21	100	0
18+	5	8	0	0
17+	0	0	1	0
16+	0	11	120	0
15+	0	7	35	0
14+	2	12	393	0
13+	0	1	86	0

TABLE 4

Neomysis franciscorum

Division of the bay	Number of		Periods of the survey					
	Hauls	Speci-mens	1	2	3	4	5	6
Upper bay............................	19		4	4	5	4	2	0
		178	27	45	30	44	32	0
Average per haul.....................		9.4	6.8	11.3	6.0	11.0	16.0	0
Middle bay..............................	44		7	13	6	6	0	8
		599	87	247	85	61	0	119
Average per haul.....................		13.6	12.4	19	14.2	10.2	0	15
Lower bay................................	53		12	11	8	3	6	11
		1078	286	160	102	31	123	376
Average per haul.....................		20.3	24	14.5	12.8	10.3	20.5	34.2

TABLE 5

Neomysis kadiokensis

Division of the bay	Number of		Periods of the survey					
	Hauls	Speci-mens	1	2	3	4	5	6
Upper bay	4		0	0	0	0	4	0
		32	0	0	0	0	32	0
Average per haul		8	0	0	0	0	8.0	0
Middle bay	24		1	10	3	4	0	6
		459	12	76	165	31	0	175
Average per haul		19.1	12.0	7.6	55.0	7.8	0	29.2
Lower bay	20		2	2	2	0	5	9
		487	2	2	260	0	139	84
Average per haul		24.4	1.0	1.0	130	0	27.8	9.3
Totals	48	978						
Average per haul		20.4						

TABLE 6

Neomysis mercedis

Division of the bay	Number of		Periods of the survey					
	Hauls	Speci-mens	1	2	3	4	5	6
Upper bay	22		3	5	5	3	3	3
		825	36	113	157	10	489	18
Average per haul		37.5	12.0	22.6	31.8	3.3	163.0	6.0
Middle bay	5		0	0	3	0	0	2
		5	0	0	3	0	0	2
Average per haul		1	0	0	1	0	0	1
Lower bay	2		0	1	0	1	0	0
		2	0	1	0	1	0	0
Average per haul		1	0	1	0	1	0	0
Totals	29	832						
Average per haul		28.7						

TABLE 7

Neomysis macropsis

OCCURRENCE IN DIFFERENT PARTS OF THE BAY AND AT DIFFERENT PERIODS
OF THE SURVEY

Division of the bay	Number of		Periods of the survey					
	Hauls	Speci-mens	1	2	3	4	5	6
Upper bay..............................	34		6	7	5	5	8	3
		6425	414	2009	403	602	1659	1338
Average per haul		189.0	69.0	287.0	80.6	120.4	207.4	446.0
Middle bay................	52		8	13	16	6	4	11
		3132	116	636	623	67	72	1618
Average per haul....................		60.2	14.5	49.0	62.3	11.2	18.0	147.1
Lower bay...............................	56		14	11	10	5	8	8
		8888	2499	478	266	747	2104	2794
Average per haul....................		158.7	178.5	43.4	26.6	149.4	263.0	349.3
Totals.........	142	18,445						
Average per haul........		129.9						

TABLE 8

Neomysis costata

Division of the bay	Number of		Periods of the survey					
	Hauls	Speci-mens	1	2	3	4	5	6
Upper bay..............................	3		0	0	3	0	0	0
		6	0	0	6	0	0	0
Average per haul................		2.0	0	0	2	0	0	0
Middle bay..............................	18		3	6	2	4	0	3
		289	39	144	6	21	0	79
Average per haul................		16.0	13.0	24.0	3.0	5.3	9	26.3
Lower bay..............................	2		0	0	2	0	0	0
		25	0	0	25	0	0	0
Average per haul................		12.5	0	0	12.5	0	0	0
Totals............................	23	320						
Average per haul........		13.9						

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FLAGELLATES OF THE GENUS TRICHONYMPHA IN TERMITES

BY

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CONTENTS

INTRODUCTION

The genus *Trichonympha,* which is the best known of the genera of hypermastigote flagellates in termites, has been the subject of a number of detailed studies, beginning with the work of Joseph Leidy (1877, 1881) on *Trichonympha agilis,* and followed by that of Grassi (1893, 1917), Porter (1897), Foa (1904), França (1916), Kofoid and Swezy (1919), Koidzumi (1916, 1921), Duboscq and Grassé (1927) and Bernstein (1928). Under other names, flagellates which have later (Duboscq and Grassé, 1927b) been placed in the same genus were described by Frenzel (1891b) and Dobell (1910). The method of ingestion of wood by *Trichonympha campanula* was studied by Swezy (1923) and Cleveland (1925a), and experimental work on this and other members of the fauna of *Termopsis* was carried out by Cleveland (1925b), Andrew (1930), and Lund (1930).

The writer undertook the study of *Trichonympha* with the intention of describing some unrecorded details of structure in the members of the genus in *Termopsis,* giving an account of certain parasites of

the flagellates, and recording several species encountered in termites from Central America and Mexico. During preparation of this paper it became clear that *Trichonympha campanula* really consists of two species, which are markedly distinct from each other; the name *campanula* has therefore been restricted to the one which corresponds more closely to the description by Kofoid and Swezy (1919*b*). Ten entozoic microorganisms have been found, six of them occurring in the species of *Trichonympha* in *Termopsis*.

In addition to the new species, the writer has been able to study all the species of *Trichonympha* described by previous authors except *T. cordubensis* (Frenzel) and *T. minor* Grassi. Consequently he has undertaken a comparative account of all members of the genus reported as occurring in termites, with the object of lessening somewhat the confusion which exists concerning the structure of these flagellates.

Acknowledgments are due to Dr. S. F. Light, for aid in the taxonomy of termites, for material from Mexico, and for most of the alcoholic specimens; to Mr. G. F. Hill, for material from Australia; to Dr. L. R. Cleveland, while collecting for whom the writer was enabled to obtain the Central American termites; to Dr. T. E. Snyder, for determination of the Central American termites; to the Research Board of the University of California for a grant in support of this research; to Miss Dorothy G. Harris, for her unusual skill and care in execution of the illustrations; to Mr. J. E. Gullberg, for preparation of the microphotographs; and to Margaret Thomson Kirby, for assistance in preparation of the manuscript.

TECHNIQUE

The study of living material has been most productive in revealing the general features of the anatomy of *Trichonympha*. Temporary preparations were used in which the living animals were placed in .6 per cent salt solution under paraffin-sealed coverglasses on ordinary slides. For the purpose of rendering the cytoplasm clearer, because freer of opaque inclusions, termites were fed for a week or more on filter paper.

For certain purposes Lugol's solution and various vital dyes, including neutral red, brilliant cresyl blue, and Janus green B were used. The dyes were dissolved in absolute alcohol and a drop of the solution allowed to evaporate on the slide before the animals were placed thereon.

Of the fixatives used, the most serviceable were the following: Schaudinn's fluid, which is excellent for general structure but inferior for the parabasal apparatus and certain cytoplasmic inclusions; Champy's fluid, which is especially useful for the parabasal apparatus; Zirkle's copper bichromate fixative, excellent for the chondriosomes and some other cytoplasmic inclusions; osmic vapor; and Bouin's fluid. Heidenhain's haematoxylin, Regaud's haematoxylin, and Delafield's haematoxylin were used after these fixatives. In some cases, the Kull method was used after Champy's fluid, and alum carmine after Schaudinn's fluid. Feulgen's method and Mallory's stain after Zenker's fluid, were used for special purposes, which are given below. Impregnation in 2 per cent osmic acid after fixation with equal parts of saturated corrosive sublimate and 2 per cent osmic acid gave results which are reported in the proper places.

The use of Zirkle's copper bichromate fixative deserves special mention. This was made up according to the formula given by Zirkle (1927), as follows:

Copper bichromate	5 gm.
Cupric oxide	1 gm.
10 per cent solution acetic acid	1 cc.
Water	200 cc.

The smears were left in the solution for the minimum period recommended by Zirkle, about 36 hours, but careful attention has not yet been given to the time of optimum fixation for this material. They were then stained with iron-haematoxylin. While the results obtained with *Trichonympha* were not especially striking, although superior for certain purposes to those obtained by any other method used, some excellent preparations have been made of several other hypermastigotes. Various intracytoplasmic fibrils have been demonstrated very clearly after use of this method. The writer feels confident in recommending further trial of the fixative for Protozoa, as it seems quite likely that it will prove a very useful addition to the technique employed in demonstration of the cytological details of ciliates and other groups, as well as of hypermastigote flagellates.

Sections of *Trichonympha* were prepared after fixing the hind-gut of *Termopsis angusticollis* in Champy's or Bouin's fluid, by cutting it entire to a thickness of five, seven, and ten microns. The sections which had been fixed in Champy's fluid and stained in Regaud's haematoxylin were especially useful for the study of basal granules and their connections, which it has been impossible to demonstrate clearly without sectioning. They were also valuable for study of the

details of structure of the parabasal bodies. Whole guts of *Porotermes grandis,* which had been fixed in Schaudinn's fluid by Mr. G. F. Hill, were sectioned, and from these most of the studies of *T. magna* were made.

During the collection of material in Central America, smears were fixed in Schaudinn's fluid, or, less frequently, Flemming's fluid without acetic acid, on circular coverglasses, which were stored in alcohol in shell vials of the proper size. These were stained after several months with Delafield's or Heidenhain's haematoxylin, or alum carmine, with counterstain in some cases of acid fuchsin, erythrosin, or Biebrich scarlet.

For the survey of the distribution of *Trichonympha* in termites, a considerable number of the insects preserved in alcohol were examined. This method yielded unexpectedly fruitful results. Even in insects preserved for ten or fifteen years, the flagellates of that genus were clearly recognizable and could be studied in much detail. In one case even the parabasal apparatus was visible. Most other genera of flagellates were not so well preserved, but if the characters are well known beforehand, it should be possible to recognize many of them.

TRICHONYMPHA IN TERMOPSIS

Trichonympha campanula Kofoid and Swezy, 1919

Hosts: *Termopsis angusticollis* Hagen. California.
Termopsis nevadensis Hagen. California.
Termopsis laticeps Banks. Arizona.

Trichonympha collaris sp. nov.

Trichonympha sphaerica (Kofoid and Swezy, 1919)

Hosts: *Termopsis angusticollis* Hagen. California.
Termopsis nevadensis Hagen. California.

Figures A and B; plates 20–24, figures 1–24; plate 25, figures 25–28; plate 26, figure 31; plate 27, figures 35–37; plate 30, figures 67–69; plate 31, figures 70–77.

OCCURRENCE

As shown in the photograph of a section through the hind-gut of *Termopsis angusticollis* (pl. 31, fig. 70), the flagellates are packed closely together, practically filling the lumen of the intestine. It was noted by Kofoid and Swezy that the smaller flagellates are limited chiefly to the region near the walls.

As regards the relative numbers of the species of *Trichonympha*, there appears to be some discrepancy among different colonies of *Termopsis angusticollis* and *T. nevadensis*. Cleveland (1925*b*) estimated the ratio of *T. sphaerica* to *T. campanula* as 1:1000; Lund (1930) gave the ratio as 1:250; while Kofoid and Swezy (1919*b*) reported that *T. sphaerica* was less abundant than other members of the association of flagellates in the gut of *Termopsis*. In termites which the writer has examined, the ratio has been higher than both of the first mentioned estimates, though showing some variation. In several termites of one colony of *Termopsis angusticollis*, the three species occurred in approximately equal numbers, while in other termites of the same colony, *T. sphaerica* outnumbered the other two species combined.

In *Termopsis laticeps*, *T. campanula* is the only hypermastigote which has been found. *Trichonympha collaris* and *T. sphaerica* were absent from the one colony which was studied, and probably are not present in this termite. In all faunated individuals of the other two species of *Termopsis*, however, the three species of *Trichonympha* are present.

<div align="center">SIZE</div>

The size of *Trichonympha campanula* was reported by Kofoid and Swezy as ranging in length from 250 to 450μ, in width from 110 to 200μ, averaging in length 350μ. They included *campanula* and *collaris* under the one species name. In order to avoid any errors from contraction, shrinkage, or abnormalities of form, living individuals of each of the two species were measured by the writer. Fifty specimens of *Trichonympha campanula* ranged from 144 to 312μ in length, and from 57 to 144μ in width at the lower end of the flagella-bearing zone, which is usually the widest part of the body. The average of these is: length, 217μ, width 85μ, with an average ratio of length to width of 2.85:1.00. Fifty specimens of *T. collaris* ranged from 168 to 360μ in length and from 72 to 168μ in width, averaging 247 × 114μ. The average ratio of length to width at the posterior end of the flagelliferous zone is 2.17:1.00. Thus *T. collaris* is somewhat larger and proportionately stouter than *T. campanula;* this fact is readily observable in living material.

In fifty specimens of each species which had been allowed to die on paraffined slides, *T. campanula* ranged in length from 105 to 245μ, averaging 153μ; and *T. collaris* from 144 to 298μ, averaging 197μ.

Flagellates allowed to die in this way cast off some cytoplasm from their posterior portions, which accounts for the smaller sizes. The measurements reported by Kofoid and Swezy, as quoted above, are too high for the average condition of *T. campanula* or *T. collaris,* although there may be extremes of large size which the writer has not encountered. Forty-one of fifty specimens of *T. campanula,* and thirty of fifty specimens of *T. collaris,* fell below the minimum length of 250μ reported by them.

The range in length of *T. campanula* of apparently normal form on fixed slides from *Termopsis laticeps* was from 130 to 300μ, thus agreeing with this species from the other termites.

Kofoid and Swezy gave the size of *T. sphaerica* as varying from 165 to 190μ in length, 160 to 185μ in width. Fifty individuals of this species in the writer's material ranged from 108 to 215μ × 70 to 132μ, averaging 165 × 89μ. The ratio of length to width, which equaled or slightly exceeded 2 to 1 in many cases, averaged 1.85 to 1.00. In material allowed to die on paraffined slides, the length of *T. sphaerica* ranged from 89 to 140μ, averaging in twenty-five cases 113μ. This species is by no means spherical, as the name implies and as was reported by Kofoid and Swezy. As first pointed out by Cleveland (1925b), it is elongated in a ratio of about 2 to 1, almost as much as is *T. collaris.*

SHAPE AND SUBDIVISIONS OF BODY

The characteristic forms of the three species of *Trichonympha* in *Termopsis* are illustrated in figure B (p. 381). The widest point is usually at the posterior limit of the flagellated region, but there is sometimes a bulge in the naked portion posterior to this. All three species usually taper more or less toward the rounded posterior end, but sometimes this portion is almost globular, owing to abnormal conditions inducing rounding up, or perhaps to an unusually large quantity of contained food.

The anterior end is tapered but does not come to a point. It ends in a rounded cap or operculum, which differs in size in the three species. The cap easily collapses, and is often collapsed in fixed material, so that in many specimens on stained slides the cap is not present in its normal shape and size. Then the anterior end may appear to be more sharply pointed than it actually is in the species. The thin, delicate membrane covers a dome-shaped structure, the base of which is seldom straight but usually concave or even funnel-shaped. The

funnel-shaped form of the base of the cap was shown by Porter in *Trichonympha agilis.* The structure is filled with a homogeneous, probably fluid substance, and into its base in the middle projects the basophilic, hemispherical granule which surmounts the rostral tube.

In degenerating material there often appears anterior to and surrounding the cap a structure which varies from a protuberance little larger in size than the original cap to a relatively large, spherical vesicle. The membrane of the original cap is present, though often collapsed, the vesicle being formed by the extrusion of protoplasmic material from within it. The wall of the outer vesicle is rather well defined, because of the granules adhering to it, and it is like the walls of spherical, protoplasmic vesicles extruded from other parts of the degenerating body.

In addition to the cap, the rostrum of *Trichonympha* consists of a central rostral tube, by which alone it is connected to the remaining portion of the body, and the collar of ectoplasm, which is separated completely from the ectoplasm posterior to it. This separation is accomplished by a circular fissure which extends inward to the rostral tube. The outer margin of the collar fits closely over the rest of the body, so that under ordinary circumstances the cleft is not visible and the impression is given that the ectoplasm is continuous. The inner portion of the cleft often is broader than the outer, so that here a conspicuous space appears. Kofoid and Swezy observed the clear area, forming ''a ring completely surrounding the tube,'' but they believed the outer surface of the ectoplasm to be continuous over it, so that this constituted a circular vacuole. In specimens of *Trichonympha campanula* placed in a strong solution of cresyl blue, so that they soon became moribund, the writer has observed the collar lifted and showing clearly that it is completely separated from the ectoplasmic portion immediately posterior to it, only the rostral tube uniting it to the rest of the body (pl. 21, fig. 3).

Measurements of the rostra of the three species, excluding those of extreme sizes, are, in microns, as follows. Length of rostrum, from tip of cap through central core: *T. collaris*, 26–29; *T. campanula*, 21–26; *T. sphaerica*, 14–15. Length of rostrum at outer edge: *collaris*, 36; *campanula*, 29–33; *sphaerica*, 14–15. Diameter of rostrum at base of cap: *collaris*, 17–20; *campanula*, 12–14 (19 in an unusually large specimen); *sphaerica*, 10–12. Diameter of body at level of posterior end of rostral collar: *collaris*, 39–41; *campanula*, 33–36; *sphaerica*, 24–26.

The rostrum is largest in *Trichonympha collaris,* smallest in *T. sphaerica,* and there is, except in individuals of extreme sizes, no overlapping in the measurements of the three species.

The anterior, flagella-bearing portion of the body, posterior to the rostrum, is, in *Trichonympha campanula,* unusually long. That is the most conspicuous feature of this species, which serves to distinguish it at once from all other described species of the genus except *T. collaris* and *T. turkestanica* Bernstein. As shown in the semidiagrammatic figure on plate 20, the photographs on plate 31, and the whole figures on the other plates, the sides of the anterior portion diverge gradually posteriorly and are slightly convex. At the posterior portion of the flagellated region the sides often spread a little. Beyond the termination of the flagella-bearing region the posterior portion of the body tapers more or less, sometimes in abnormal specimens coming to a point, but usually it is broadly rounded. In the region between the two portions a constriction is apparent in the endoplasm, and sometimes the body seems superficially divided into two segments.

The ratio between the anterior, flagellated region and the posterior, non-flagellated region varies in different individuals of the same species. In twenty-five living specimens of *T. collaris,* in which the shape seemed to be normal, the ratio of anterior to posterior region varied from 0.74:1.00 to 2.14:1.00, averaging 1.32:1.00. In *T. campanula,* the flagellated zone is relatively longer, the ratio ranging from 1.34:1.00 to 2.49:1.00, averaging 1.80:1.00. In *T. sphaerica,* the anterior portion is always much less extensive than the posterior portion, the ratio ranging from 0.30:1.00 to 0.50:1.00, averaging 0.40:1.00. These ratios, of course, vary according to the degree of rounding up of the body, whether owing to conditions of preparation for observation or to normal changes in form during the life-histories of the flagellates, as at the onset and just after division.

Duboscq and Grassé (1927) reported small forms of *Trichonympha chattoni,* called *Leidyopsis*-forms, in which the anterior, flagellated region of the body was much shortened and the posterior portion rounded. These, they claim, are individuals which have just resulted from division of a larger form, and they suggest that *Trichonympha sphaerica* may be a "*Leidyopsis*-form" of *T. campanula.* There is not the slightest doubt, however, of the validity of *T. sphaerica* as a separate species in *Termopsis.* All forms of *T. chattoni,* in the shape of the body and the ratio between the flagellated and non-flagellated zones, resemble *T. sphaerica* much more closely than they resemble the other two species in *Termopsis.*

SURFACE RIDGES AND FLAGELLA

The surface of the flagella-bearing portion of *Trichonympha* is marked by longitudinal, rounded ridges. The diagrammatic optical section of *T. campanula* on plate 21, figure 4, was drawn from an animal in which the body was bent so that the edge could be seen at about the level of the middle of the prenuclear area. Plate 21, figure 5, shows these ridges on an actual cross-section of the body of *T. campanula*. The surface ridges are low and rounded, and the flagella emerge from the grooves between them. Between the ridges, extending deeply into the ectoplasm, are refractive, stainable, plate-like structures. In stating that the ridges are high and narrow, and that the flagella arise from their summits, Kofoid and Swezy had reference to these plates, which are more conspicuous than the ridges themselves. It is the plates between the ridges which are seen as longitudinal striations when one focuses upon the surface layer of the living, as well as the stained animal.

In one case forty-nine ridges were counted on the rostrum of *T. campanula* (pl. 21, fig. 6) ; in another forty-three; in another thirty-six. These end at the posterior boundary of the collar-like part of the rostrum (pl. 21, fig. 3). On the body posterior to this the ridges are about twice, or more than twice, as numerous, and consequently in their anterior portions they are closer together than those on the posterior portion of the rostrum. In the specimen with forty-nine rostral ridges there were ninety-eight body ridges (pl. 21, fig. 6) ; in others ninety-seven, one hundred, and one hundred and twelve were present. On *T. collaris* the number of ridges is about the same; one hundred and six were counted in a cross-section of the body of this species.

The thousands of flagella, which emerge in longitudinal rows between the ridges, are uniformly distributed over the anterior portion of the body. In *T. campanula* and *T. collaris*, in which the flagella are similar in size and arrangement, flagella of moderate length (about 30–60μ) arise from the rostrum. There is a marked break in the zones of flagella between the posterior end of the rostral collar and the anterior portion of the body region, where they are about 20–25μ long. Most of the flagella-bearing body region is covered by flagella of uniform length, but near the end of this region they become markedly longer, increasing to more than 150μ. The long flagella cover the posterior portion of the body, and extend a considerable distance beyond it.

In *Trichonympha sphaerica*, the flagella increase gradually in length from the anterior part of the rostrum, where they are moderately short, to the posterior limit of the flagelliferous zone, where they may be 150μ long, enough to reach beyond the posterior end of the elongated body.

ECTOPLASMIC REGION

In the rostrum and the anterior portion of the body region of *Trichonympha,* the ectoplasm is very thick. In *T. campanula* and *T. collaris* (fig. B, 1, 3; pl. 20), this thickness decreases posteriorly in the flagella-bearing region, until it is rather suddenly reduced to the thin layer of ectoplasm which covers the naked posterior portion of the body.

The ectoplasm of the flagelliferous body region of *T. sphaerica* is of even thickness from the anterior end behind the rostrum to the posterior limits (fig. B, 2; pl. 26, fig. 31). Within it the endoplasm forms a convex conical or dome-shaped structure, and at the base of the cone extends almost transversely outward, so that the posterior demarcation of the thick ectoplasm is much more conspicuous than in the other two species. Along a transverse axis of the body in this region, about half the thickness is ectoplasm and half endoplasm.

At the inner limit of the ectoplasm the layer of basal bodies of the flagella is situated, and the entire ectoplasm of the flagella-bearing region is traversed by the roots of the flagella. In this portion, as previously described in this and other species of *Trichonympha,* the ectoplasm is differentiated into layers. Kofoid and Swezy defined these layers as (1) the ridges, (2) the alveolar layer, and (3) the inner ectoplasmic layer. The three layers are designated in this paper as the outer, middle, and inner layers of ectoplasm.

The outer layer constitutes half or more of the thickness of the ectoplasm. This region is traversed by the plates, which lie beneath the grooves between the surface ridges. The plates are refractive, stainable with iron-haematoxylin, and evidently consist of dense protoplasm. Their thickness (pl. 21, fig. 5) is considerably greater than that of the roots of the flagella, which pass through the plates. The flagellar roots in the plates can be seen in living and fixed flagellates, but not so clearly as in the middle layer of ectoplasm. They run obliquely posteriorly as they pass outward from the inner region of the plates.

Posterior to the rostral fissure, in the anterior portion of the flagella-bearing region of the body, there are, in the three species,

refractive and stainable bands of denser protoplasm extending from the plates to the innermost part of the ectoplasm (pl. 21, figs. 2, 7). These possibly represent extensions of the plates inward, so that the plates become associated here with the deeper parts of the body. The bands may be optical sections of a disc of dense protoplasm.

Beneath the outer layer of the ectoplasm is a clear zone, which has been called by several authors the inner layer, but which is designated here as the middle layer. In the rostrum this layer is clear, but apparently rather dense, as it is usually very difficult to follow the flagellar roots through it. In the anterior portion of the body proper it is a fluid, as first reported by Grassi. This layer is traversed by the roots of the flagella, which are clearly visible in living and stained animals, so that it has a cross-striated appearance.

That the protoplasm of the middle layer is fluid in the body region has been determined by study of living material of *T. campanula*. Often, in this layer, between the flagellar roots, there are small granules which stain with cresyl blue and neutral red. The granules vary in abundance; in most cases they are absent, or few in number and restricted to the posterior portion; but sometimes they are numerous throughout the layer. Movements of these granules can be observed, and as the animal bends the whole group may be shifted forward or backward. In the flagella-bearing body region, then, this region is fluid-filled and is traversed by the more highly refractive roots of the flagella, which can be easily seen; in the rostrum its substance is denser, of about the same density as the roots of the flagella.

The innermost layer of the ectoplasm, which includes the layer of basal granules, is thickest in the rostrum, where it begins behind the hemispherical granule which surmounts the rostral tube, and is of practically uniform thickness to the circular fissure. It is much denser in consistency than the middle layer. In the body region it gradually diminishes in thickness in *T. campanula* and *T. collaris*, but it apparently continues to the end of the flagelliferous zone, constituting a denser protoplasm in which the basal granules are imbedded. In *T. sphaerica* the layer does not decrease in thickness (fig. B, 2), but to the posterior limit of the flagella-bearing region is as thick as in the rostrum. In *T. collaris* it is thicker in the anterior part of the body region than in *T. campanula*.

Of the three layers of ectoplasm, the middle, clear one disintegrates first. When specimens are kept until dead on paraffined slides, this

layer completely breaks down, the substance becomes very fluid, and the flagellar roots disintegrate into many minute granules which show active Brownian movements. The inner and outer layers, meanwhile, remain intact, with the roots of the flagella persisting in them.

BLEPHAROPLAST AND ROSTRAL TUBE

Reference has been made in the preceding account to the hemispherical granule which lies in the base of the middle of the cap. The separation between the cap and the rostrum coincides with the flat base of this granule, and the ectoplasmic layers of the rostrum converge toward it (pl. 21, fig. 2). The outer border of the inner layer is continuous with its margins.

The granule, which is shaped like a split pea, is highly refractive and is easily seen in living specimens of the three species. It stains well with Delafield's haematoxylin, with which it, as well as other structures of the rostrum, can best be demonstrated in preserved material. It stains with iron-haematoxylin, but loses its blackness when the material is differentiated sufficiently for other structures. Its basal portion, however, retains the stain more tenaciously, especially at the points where the siderophile rostral tube joins it. Here there may appear, in optical section, to be two granules. Kofoid and Swezy overlooked the structure but observed the especially siderophile basal ring as a "circular band." Some of their figures, however, show a structure at the top of the rostral tube which corresponds in size and form to this granule, whereas the cap is omitted (their pl. 5, figs. 2 and 6).

Through the center of the rostrum passes the rostral tube (pl. 21, fig. 2; pl. 31, fig. 71). It is refractive, so that it can be seen in living animals, and it stains readily with haematoxylin. It is constricted just beyond the point where it joins the anterior granule, and spreads at its posterior end where it continues into the next portion of the body. Within the rostral tube is a core of endoplasm, and only by this core and the tube is the rostrum joined to the rest of the body. The flagellar roots extend inward to the rostral tube, which doubtless consists of fused basal granules. Kofoid and Swezy, who call this structure the centroblepharoplast, describe it as composed of separate strands, but it is really a tube with solid walls.

In the species of *Trichonympha* in *Termopsis*, there has not been found any structure corresponding to the ring surrounding the base

of the rostral tube described by Bernstein as a sphincter in *T. turkestanica*. She believed that the sphincter serves to close the tube and to prevent the granules of the endoplasm passing into the tube. In the species which the writer has studied, there is no evidence that the endoplasm is not continuous into the lumen of the tube, and there are granules in the tube as well as elsewhere. Furthermore, no constriction of the base of the tube has ever been observed in these species, and none is reported by Bernstein in her species.

BASAL BODIES OF FLAGELLA

At the inner ends of the flagellar roots are structures which may be homologized with the usual basal granules, but which are unusual in the three species of *Trichonympha* in *Termopsis* in that they are rodlets (fig. A, 2–5). The rodlets are arranged in longitudinal rows corresponding in number with the plates, and also in closely set transverse rows (pl. 20, fig. 1). The direction of the rodlets themselves is neither longitudinal nor transverse, but diagonal. Thus there is a third set of rows, following the long direction of the rodlets, slanting from posterior right to anterior left in a laeotropic spiral. Furthermore, the rodlets are not parallel to the surface of the animal, but oblique, so that at the upper level in an animal with the longitudinal axis horizontal their right ends are nearer the body surface (fig. A, 3). The roots of the flagella meet these ends (fig. A, 2, 3, 5).

The rodlets themselves are interconnected in two directions by delicate filaments. The ends opposite to those which are connected to the flagellar roots are joined to one another, in the longitudinal direction of the body, by very slender filaments (fig. A, 2). The same end of each rodlet is also connected to the opposite end of the rodlet in the same diagonal but next longitudinal row by a filament (fig. A, 5). This filament leaves one rodlet close to the point where it is joined by the longitudinal filament, and meets the other rodlet close to its junction with the flagellar root.

The structure, size, and arrangement of the basal rodlets appears to be the same in *T. campanula* and *T. collaris,* in which some of them are one to one and one-half microns long. In *T. sphaerica* there are similar, though shorter, basal rodlets, but it is much more difficult to study them in that species than in the others.

As one focuses downward on a whole *Trichonympha,* one first encounters the ridges, then the plates beneath the grooves between
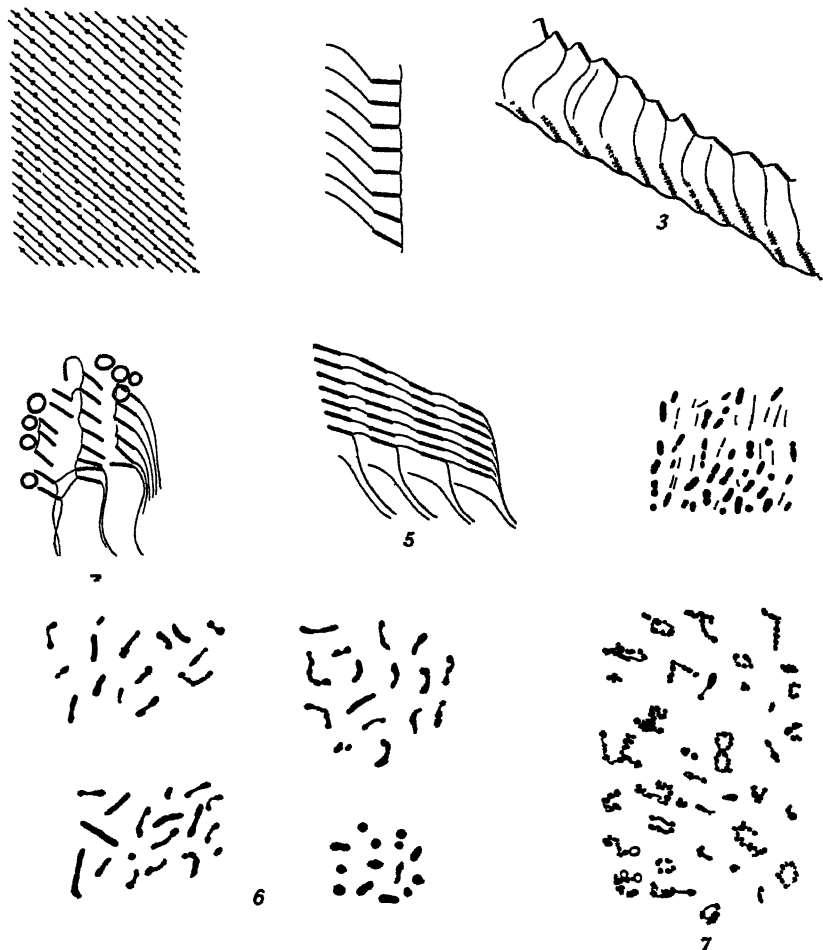
Fig. A. 1–7 from *Trichonympha campanula.* 1. Diagram showing the diagonal lines visible in living material and preparations when the microscope is focused upon the deepest layer of the ectoplasm. The black dots represent sections of the roots of the flagella, and appear at different levels as the microscope is focused up. The figure does not represent the actual structure, but is a fairly exact representation of the optical appearance in whole mounts. 2. Basal rodlets, roots of the flagella connecting to the left end of each and longitudinal filaments connecting the other ends. Ch. R. 3. Basal rodlets, showing diagonal connecting filaments, plates and roots of flagella, and indicating that in their normal position the rodlets do not lie in one plane but that each is oblique to the inner ends of the flagellar roots. Ch. R. 4–5. Basal rodlets, showing roots of flagella (below and on right), diagonal filaments connecting distal end of one rodlet with proximal end of another, and longitudinal filaments. Ch. R. 6. Chondriosomes; those in the group at the lower right are abnormal because of poor fixation. Z. H. 7. Forms of groups of anterior endoplasmic granules observed in preparations fixed by this method. Z. H. 8. Peripheral granules from *T. collaris.* Apparent division of some, and slender rods among them. 1–5 are based on camera lucida drawings × 2000. Others are free-hand sketches. Abbreviations: Ch. R., Champy's fluid and Regaud's haematoxylin; Z. H., Zirkle's copper bichromate fixative and Heidenhain's haematoxylin.

the ridges. These appear as refractive, or in certain preparations stained, parallel stripes, between which are clearer areas. The dense plates can be followed down for a certain distance. They are deeper near the anterior end, becoming shallower near the posterior part of the flagellated region. Then one focuses into the clear area, in which the roots of the flagella at the upper level appear in optical section as granules arranged in diagonal, longitudinal, and transverse lines. As the roots of the flagella do not run vertically, but obliquely to the surface, and as the image is somewhat confused by diffraction patterns, the result of a superficial examination is a conception of a more intricate pattern of fibrils than actually exists. Below this region, the middle layer of ectoplasm, the basal rodlets come into focus. As they lie obliquely to the surface, they appear in optical section as small granules, but since they are actually rods they can be seen at a series of focal points. At the deepest focal point appear fine diagonal lines, running from posterior right to anterior left. Figure A, 1, represents the picture obtained in a focus at this level on some material stained in iron-haematoxylin, but it is certain that this picture does not represent the actual conditions. One does not get in whole mounts a clear image of the basal rodlets and their connections; in order to study them it is necessary to use sections.

The diagonal striation deserves special mention, as it is a conspicuous feature of many specimens of *Trichonympha campanula* and *T. collaris*, and is liable to misinterpretation. In many flagellates, prepared by a variety of methods, or even in living material not stained at all, diagonal striations are conspicuous, running from posterior right to anterior left on the upper side, and in the opposite slant on the lower side of the animal. The angle is usually about ·45 degrees to the longitudinal axis, and often is inclined toward the longitudinal more than this, but sometimes the striations are almost transverse. At first glance, these appear to be continuous lines, but closer analysis shows that this is not the case. Actually the lines are broken, and they are produced by the basal rodlets which lie in rows in a laeotropic spiral direction and whose ends are at no great distance from one another, if, indeed, they do not lie over one another. That this is the case can be determined with no possibility of doubt by study of longitudinal sections of *Trichonympha*, where the same diagonal striations may be seen, but it is clear that the basal rodlets are responsible for this appearance.

The striations described above are, of course, at the innermost part of the ectoplasm. Excepting these, no striations which approach the

transverse have been observed in any material at the disposal of the writer. It has not been possible, consequently, to verify the existence of transverse myonemes in *Trichonympha campanula*.

VACUOLIZATION OF ECTOPLASM AND DISARRANGEMENT OF PLATES

When *Trichonympha* is properly prepared, the surface of the flagella-bearing region appears longitudinally striated by close-set, parallel, or anteriorly converging lines, which are the stainable plates. The middle layer of ectoplasm is homogeneous except for the striation resulting from the great numbers of flagellar roots which traverse it beneath the plates.

When the animals are not properly prepared, the appearance is often quite different from this, resembling the condition shown in the photograph (pl. 31, fig. 77). In this specimen, which occurred near the edge of a smear on which were many normal flagellates, prepared by the Schaudinn-Delafield technique, the plates below the surface ran parallel to one another, but the middle zone of ectoplasm was occupied by a layer of vacuoles. The vacuoles were of diverse sizes and irregular arrangement, small ones intervening between larger ones. The vacuoles were packed closely together, and the spaces between them were darkened by the stain.

On the same slide, also near the edges of the smear where the conditions of preparation were not such as to give normal specimens, flagellates were found in which the number of these vacuoles was less, their size was smaller, and they did not all touch one another. In some cases there were only a few small vacuoles, restricted to the extreme posterior region of the flagellated zone. In normal specimens, nothing is to be seen of these vacuoles, and from the variability in their development it is clear that the alveolar zone is not a characteristic, normal structure of *Trichonympha*. Under certain conditions, vacuoles develop in the fluid layer of the middle zone of ectoplasm, among the flagellar roots, which may be displaced by them, and the number of these may increase until they form a continuous layer of alveoli. This, in its complete development, doubtless is the alveolar layer described by Kofoid and Swezy.

When the animals are placed in salt solution of too great concentration (.9 per cent or 1 per cent NaCl), they immediately become motionless, and numerous vacuoles, of diverse sizes and irregular arrangement, appear in the middle ectoplasmic layer of the flagelliferous zone. Some flagellates develop a continuous alveolar layer of the

degree shown in the photograph (pl. 31, fig. 77). In those which recover their activity under a coverglass, as most of them do in time, the vacuoles gradually disappear. Their development can be induced by placing *Trichonympha* in a hypertonic medium, and the more hypertonic it is, up to a certain point. the more vacuoles develop. Normal salt solution quickly induces their appearance. An isotonic or hypotonic medium does not cause development of the vacuoles; even distilled water, which kills the flagellates, does not induce vacuolization. Of sodium chloride solutions, one with a concentration of .55 per cent was found most favorable for *Trichonympha*; even in .6 per cent NaCl, which has been used a great deal by the writer for the purpose of dilution of the intestinal contents of *Termopsis*, some vacuoles develop in many of the flagellates. If physiological salt solution is used as a medium for dilution, and the flagellates fixed a few moments after teasing them out, the vacuolated condition of the middle zone of ectoplasm will appear in part of the preparations.

In some specimens on the slide from which the photograph (pl. 31, fig. 77) was made, in which vacuolization had occurred, it was observed that the plates did not follow directions parallel to one another, but were very sinuous so that in places adjacent plates came together. The result was what would have been the case if the body contracted longitudinally and caused a sinuosity of non-contractile plates. The plates are always straight in normal specimens, however. If their unequal sinuosity in the above cases is a result of contraction, that must be a contraction which is not normal, and which possibly is the result of immersion in a hypertonic medium.

The disarrangement of the plates was shown most clearly on a slide loaned to the writer by Dr. Virginius E. Brown, which had been prepared by an aluminum chloride-osmic acid technique after smearing in normal salt solution. There had been, in various specimens, different degrees of vacuolization of the ectoplasm, and the walls of the alveoli, or spaces between them, were darkened as a result of the technique used. The plates were darkened in a similar manner, but the preparation was not favorable for study of any other details of the structure of *Tricho-nympha*. Some specimens were quite normal in form. In them the plates ran straight and parallel or smoothly converging, and there were very few or no vacuoles in the ectoplasm. At the other extreme there was a high degree of vacuolization, like that shown in the photograph, and a more or less extreme sinuosity of the plates. In producing the latter condition, two adjacent plates successively spread apart and came

together, enclosing between them areas irregularly lens-formed, which alternated with similarly formed areas enclosed between each of these plates and the next outer ones. The picture resulting from this arrangement is that of a network of coarse, and in this case, very dark fibers. The general arrangement of the apparent network is similar to that of the oblique fibers shown by Kofoid and Swezy (1919b, pl. 6, fig. 7; pl. 8). If, however, one studies carefully the nodes of this apparent network, one sees that the fibers, or rather plates, do not interlace nor join, but that here, as described above, two adjacent plates merely are approximated. There are cases where they do not meet at the position which corresponds to the nodes of the network elsewhere. In some specimens, over portions of whose bodies the apparent network extends, there are parts where the plates are straight and parallel, or nearly so; and it may often be observed that the straight portions are continuous with the sinuous portions.

Directly beneath the disarranged plates lie the vacuoles which have developed, in such cases, in the middle layer of ectoplasm. When one focuses down upon these, there comes into view a continuous reticulum constituted by the darkened interalveolar regions. There appears to be some degree of correspondence between the spreading of the plates and the development of vacuoles, as if the spreading were associated with the extension of vacuoles between the plates, forcing them apart.

Vacuolization of the middle zone of ectoplasm and disarrangement of the plates occurs in all three species of *Trichonympha* in *Termopsis*, but the above account deals with *T. campanula* and *T. collaris*, in which these abnormalities can be studied more easily than in *T. sphaerica*.

PERIPHERAL GRANULES

The peripheral granules are small granules located in the outer zone of ectoplasm of *Trichonympha collaris*, both in the rostral and body region, between the plates through which the roots of the flagella pass. They are not present in *T. campanula* or *T. sphaerica*, and therefore serve as a ready means of distinguishing *T. collaris*.

In form, the peripheral granules vary. The majority are short, blunt rods, but many are rounded or slightly elongated granules. Many are constricted, some quite completely separated into two. It is likely, therefore, that these granules multiply by division. Among them, sometimes, are some very slender rods of variable length (fig. A, 8).

They are most numerous in the outer layer of ectoplasm in the posterior half of the rostrum, where they are densely packed to the posterior limit of the part which overhangs the flagelliferous region of the body. Just behind the fissure peripheral granules are not present, but a short distance beyond they recur and often occupy the whole region from this point to the posterior limit of the flagella-bearing zone. In *T. collaris,* they are quite constant in occurrence, abundance, and distribution.

The granules are clearly visible in unstained material, and can be stained with iron-haematoxylin after Schaudinn's fluid and other fixatives. They quickly lose the stain, however, when the body is differentiated sufficiently for detailed observation of internal structure. They do not stain with neutral red. In certain material prepared by the Feulgen-light green method, the peripheral granules stained purple, and by their presence it was possible to trace the course of the ridges, which otherwise could not be seen.

The rostrum in *T. collaris* has the appearance of a collar (pl. 22, fig. 12) striped with granular bands, and is sharply demarcated from the portion of the body posterior to it. It is possible that the rows of granules in Duboscq and Grassé's text figure 6 (1927b), which are taken to be the basal granules, are peripheral granules. They are too uneven in their distribution and the rows are not properly spaced for basal granules.

The peripheral granules resemble in form the granules given this name by Peschkowskaya (1928) in *Climacostomum virens* and by Studitsky (1930) in *Dileptus gigas.* In *Dileptus gigas,* constricted granules, which apparently are dividing, were described, and Studitsky reported that intensive epidemics of division of the granules occur during times of reproduction of the ciliates. Though it does not seem likely that the structures are identical in *Trichonympha* and the ciliates, the resemblance between them is striking.

Granules identical in form and distribution with the peripheral granules of *Trichonympha collaris* were described by Bernstein in *T. turkestanica,* as discussed below (p. 389).

ACTIVITIES

The movements of the three species of *Trichonympha* in *Termopsis* are similar, save for diversities due to differences in the build of the bodies. The animals when in normal condition are very active, and presumably, when undisturbed in the hosts, their movements are not interrupted at any time. In preparations made for examination of living animals, the activities cease entirely when the flagellates are first put into an unfavorable medium, such as normal salt solution, but after a time their power of movement usually is regained.

The rostrum, and with it a variable portion of the anterior body region, moves irregularly from side to side, or bends back against one side of the body. These bending movements in *T. campanula* are illustrated by Kofoid and Swezy (1919*b*, text fig. C). The animal performs such movements erratically, at irregular intervals when it is moving forward, or when it is active though held in one place. The anterior portion then often bends backward until it comes to lie in a concavity of the body, after which the animal may remain quiet for a moment, then suddenly straighten out again and begin normal activity. In *T. sphaerica*, the backward bending of the rostrum is accompanied by a shoulder-like protrusion of the portion of the body posterior to the concavity in which the rostrum comes to lie. Because of the build of the body of *T. sphaerica*, the shoulder-like protrusion is a conspicuous feature of it and not of the other two species. In *T. collaris*, the anterior end may be caused to describe a circle, the direction of which, as viewed from the posterior of the animal, is clockwise.

The rostrum moves from side to side as a unit, without any change of form, the bending taking place behind it, where the body is very mobile. The dense transverse bands just behind the rostrum also move solidly, but the ectoplasmic plates may be caused to bend outward. The middle zone of the ectoplasm, in the anterior part of the body, may be compressed on the side toward which the rostrum bends, and the angle at which the roots of the flagella pass through the zone may be altered. This is another evidence of the fluidity of this zone of ectoplasm. A disturbance which takes place in the endoplasm of the anterior portion of the body may extend to the nucleus, the shape of which is sometimes thereby altered.

When the bending movements are slow enough to be analyzed, it may be observed that alternate strokes of the flagella accompany them. A bending of the rostrum to one side is accompanied by a

vigorous forward stroke of the rostral flagella on that side, and the return bend follows the backward stroke of the flagella. Opposite movements occur in the flagella on the other side. More extensive activity is accompanied by similar strokes of the short body flagella.

The movements which occur when activity is restricted enough to be followed can, it seems, be accounted for entirely through the strokes of the flagella. It seems to the writer likely that more vigorous movements can be accounted for in the same way, since flagellar activity increases at the same time, and that it is not necessary to assume the existence of myonemes to account for the activities of *Trichonympha*. The writer has seen no evidence of the existence of longitudinal myonemes, other than the ectoplasmic plates which have been called myonemes by some authors (Grassi, Duboscq and Grassé), and no constriction of the body such as would follow the action of transverse myonemes has been observed to occur.

In all three species of *Trichonympha* a vigorous vibration of the periphery of the body often occurs. This vibration seems to consist of undulatory waves which pass backward, spreading to the non-flagellated portion of the body, and which are probably caused by the beating of the body flagella. Forward locomotion is usually accompanied by vigorous vibrations of the sides in this way, but the animals can glide forward without such vibrations. Both types of movement, the vibration of the sides and the bending of the rostrum, may occur without locomotion.

As the flagellate moves forward, its body revolves, if it is not confined too closely under a coverglass, in a direction from its right over to the left. This turning, of course, is very uneven in consequence of the jerky movements. Sometimes an animal, which is cornered and therefore unable to move forward, but nevertheless is free to turn, revolves at an even rate in this direction as a result of the movements of the flagella.

The most active flagella are those of the rostrum; less active are the short ones of the body. The long body flagella, which lie against the surface with their terminal portions extending beyond the posterior end, are vibrated slightly or not at all. These do not, under ordinary circumstances, leave their position against the body surface.

PARABASAL APPARATUS

The parabasal apparatus of *Trichonympha* consists of a large number of slender cords similar to those described by Duboscq and Grassé in *Trichonympha chattoni*. It has not been possible in *T. collaris* and *T. campanula* to trace the parabasal cords to the base of the rostral tube, though in *T. sphaerica* they have been followed nearly to this position. In *T. campanula* they first appear in the peripheral endoplasm about halfway between the nucleus and the rostrum, and they terminate near the posterior end of the flagellated region (pl. 22, fig. 8), a short distance beyond the nucleus. In this species the parabasals often form a curtain-like structure around the nucleus. In their portions posterior to the nucleus, and often throughout their extent, the parabasal cords of *T. campanula* are usually sinuous; rarely are they straight; their posterior ends are often strongly curved or hooked. They are not parallel to the longitudinal axis of the body, at least in many cases, but are turned in a long, laeotropic spiral. In all cases some of the parabasals bend inward toward the nucleus and are attached along or at least lie adjacent to the sides of the nuclear membrane for a short distance (pl. 22, fig. 9) before continuing.

While in *T. campanula* the parabasal cords are separate from one another and quite evenly distributed in the endoplasmic region around the nucleus, in *T. collaris* they all converge inward toward the nucleus, running either against the membrane or close to it (pl. 22, fig. 12). Also, in *T. collaris*, they extend farther into the postnuclear endoplasm, and in their posterior portions are collected into groups of two to six or more. The number differs in different groups, and there are some single ones as well, but most of the cords in this species of *Trichonympha* are so united. Plate 22, figure 11*b* shows a cross-section of a group of seven. In the region behind the nucleus the groups are sometimes independent of one another, sometimes intertwined. This difference in the arrangement of the parabasal cords is a constant difference between the two species.

Anterior to the nucleus the parabasals in certain preparations of both *T. campanula* and *T. collaris* appear to be distributed around the periphery of an ellipsoidal or bowl-shaped area which is relatively clear. This extends from the nucleus, which lies at its bottom, to the periphery of the endoplasm about halfway to the rostrum. It corresponds to the "corbule" as described by Koidzumi, to whom the layer of parabasals, not seen as such, suggested a membrane which

sometimes had longitudinal, band-like thickenings. Actually, however, there is not a membrane, and the effect of a corbule is not apparent in all material.

The parabasal apparatus of *T. sphaerica* (pl. 26, fig. 31) consists of numerous separate cords beginning near the posterior end of the rostral tube and ending a short distance beyond the nucleus, where they are often strongly curved or hooked, as in *T. campanula*. Cords have not been observed to lie against the nuclear membrane, as is the case in the other two species.

The parabasal apparatus has been demonstrated most clearly after fixation in Champy's fluid and staining in Regaud's haematoxylin. After Flemming's fluid without acetic acid the cords take a brown stain with iron-haematoxylin, like the parabasal body of *Trichomonas termopsidis* on the same slides. With Mallory's stain they become blue, and after the Champy-Kull method brown, in both cases also like the parabasal of *Trichomonas*. After Schaudinn's fluid with acetic acid they usually do not stain well, but they do not dissolve, as Duboscq and Grassé claim, but can be demonstrated clearly both by Delafield's haematoxylin and Heidenhain's iron-haematoxylin. In material stained with alum carmine after this fixative they are often visible, though unstained. Furthermore, they may be stained, though not very well, by iron-haematoxylin after fixation in the vapor of glacial acetic acid. They are visible in living, unstained material, and are rendered very distinct, though not at all blackened, after the flagellate has been immersed for some time in osmic acid. In their chemical reactions, then, these cords resemble the parabasal bodies of the Trichomonadidae, especially of *Trichomonas termopsidis* and the Devescovininae.

In their structure the parabasal cords correspond in general to those of *Trichonympha chattoni* as described by Duboscq and Grassé (1927). They are considerably longer than these, and they cannot be observed to end abruptly at a point of attachment to a free parabasal filament, but decrease gradually in size to the point where they are lost to view. The outside region of the cords stains most readily with iron-haematoxylin, as indicated in the sections and flat views of the bodies stained with iron-haematoxylin after Schaudinn's fluid (pl. 22, fig. 11 *b*, *c*). In material prepared by the Champy-Regaud method a structure like that shown in *T. chattoni* is visible. There is a chromophile filament along one side, and the remainder is a chromophobe part containing clear vesicles (pl. 22, fig. 11 *a*). Accord-

ing to the writer's observations, the outlines of the chromophobe part are not uneven, as they are described in the other species, but the parabasals are smooth cords. The writer has found no evidence of vesicles being released from the parabasals, as claimed by Duboscq and Grassé, though granules similar in size to those which they depict have been seen lying in contact with the cords.

NUCLEUS

There are characteristic differences between the nuclei of the three species of *Trichonympha* in *Termopsis* by means of which it is possible to assign them to the proper species without consideration of other structures. That of *T. campanula* (pl. 30, fig. 68) is spheroidal or ellipsoidal, from 18 to 33μ in diameter in the former case, from 13×20 to $29 \times 36\mu$ in the latter, and it is situated in the region enclosed by the base of the flagellated zone, thus being near or posterior to the middle of the body. Between the central chromatin mass and the membrane is a space more or less wide according to the degree of contraction of the mass, but the space is present even in living material. The chromatin is not divided up into separate granules on a linin reticulum; instead there are rather slender, much coiled, varicose strands irregularly arranged and interconnected in places by chromatic filaments. No linin reticulum has been distinguished. From the chromatin mass outward toward the membrane there often extend some slender filaments. Sometimes these appear to mark off around the chromatin, spaces which are liable to misinterpretation as alveoli. In the region just beneath the membrane are many fine granules stainable with iron-haematoxylin, but not with alum carmine or Feulgen's stain, forming the granular area described by Kofoid and Swezy.

The most characteristic thing about the nucleus of *T. campanula*, by which it can be distinguished at once from the nuclei of all other species of *Trichonympha* examined in preparation of this paper, is the "heterochromosome" described and figured by Kofoid and Swezy. This is situated in a clear space close to the nuclear membrane. It has not been possible for the writer to verify the existence of a membrane around the apparent vesicle. The "heterochromosome" is variable in form; sometimes it is a short, coiled rod, sometimes a slightly curved to sharply bent rod, sometimes it is broken up into parts, but it is never round or ellipsoidal like the similarly situated body in several other species of the genus. It probably consists of chromatin, not

plasmosome material, for it stains like chromatin with Feulgen's stain, Delafield's and Heidenhain's haematoxylin, Mallory's triple stain, and alum carmine. The writer has not followed this body through the mitotic process, but according to Kofoid and Swezy it remains isolated throughout division and divides at or before the metaphase.

In *T. collaris* the nucleus (pl. 30, fig. 69) is larger than in *T. campanula*, measuring from about $26 \times 26\mu$ to $30 \times 35\mu$. It is situated in a comparable relation to the flagellated zone, but is more anteriorly placed because of the more restricted extent of this zone. During bending movements of the living animal the shape of the nucleus has been observed to undergo repeated alterations. Whether or not this is due to the traction of the parabasal cords could not be decided, but it may be due merely to disturbances of the endoplasm. The central chromatin mass usually fills the space within the membrane, save for a narrow clear area in which are some small granules and radial filaments. The chromatin is formed into coiled and interconnected strands, which are stouter and somewhat more compactly arranged than in the preceding species. No nucleolus has been recognized; certainly there is nothing like the "heterochromosome" of *T. campanula* or the large nucleolus of *T. chattoni.*

The nucleus of *T. sphaerica* (pl. 30, fig. 67) ranges from about 19 to 30μ in diameter. In general appearance, the presence of fine granules in the clear area and the arrangement of chromatin, it is similar to the nucleus of *T. campanula.* No "heterochromosome" or conspicuous nucleolus is present, however, though in many cases there was detected a small rounded or elongated body peripheral to the main mass of chromatin. This stained like chromatin and sometimes appeared to be connected by a filament to the central chromatin mass.

ENDOPLASM AND ITS INCLUSIONS

MINUTE ENDOPLASMIC GRANULES

As may be observed in living material, the endoplasm in both prenuclear and postnuclear regions contains a great number of minute, closely packed granules. The prenuclear region is of uniform, finely granular texture, save for the larger granules which are described below. In the postnuclear endoplasm there are usually many wood particles and a number of small spherules and vacuoles, but the ground substance of the cytoplasm is of the same finely granular texture.

There is no evidence of an alveolar structure, besides that due to the food vacuoles.

Slight Brownian movements of the minute endoplasmic granules of the posterior region can sometimes be seen in active, living flagellates, while the prenuclear granules are stationary. Evidently, the postnuclear endoplasm is the more fluid.

These granules do not stain with neutral red. With Lugol's solution they stain yellow or yellow brown, while the ectoplasm becomes pale yellow. As this is regarded as a glycogen reaction, it seems that the minute granules are glycogen, or at least are glycogen-like. It is these granules, then, that give the glycogen reaction in *Trichonympha* (see p. 437).

CHONDRIOSOMES

Chondriosomes have been described by Duboscq and Grassé in *Trichonympha chattoni*. Bodies similar to them in form and distribution occur in *Trichonympha campanula*, *T. collaris*, and *T. sphaerica*. They have been demonstrated best by iron-haematoxylin after Zirkle's fixative, and by the Champy-Kull technique. They have been shown, but not so well, by Regaud's haematoxylin after Champy's fluid, and they are visible after iodine staining, standing out sharply against the yellow or brown background of minute granules. The writer has not succeeded in staining them with Janus green B. This has been attempted in 1:10,000 solution in .6 per cent NaCl, and in 1 per cent solution in absolute alcohol dried on the slide. Janus green appears to penetrate little if at all into *Trichonympha*. In living animals the wood particles do not stain, as do those outside the Protozoa and as do some in the bodies of *Trichomonas*.

Chondriosomes are very abundant in the endoplasm of the posterior portion of the body, and they are quite evenly distributed in this region, except for the spaces occupied by the ingested wood (pl. 20, fig. 1; pl. 23, fig. 16). Often, however, they are more numerous than usual in the regions where the parabasal cords of *T. campanula* end (pl. 22, fig. 8). Among the parabasals in the vicinity of the nucleus they are few, but in the narrow zone of endoplasm outside of the posterior portions of the parabasals they are numerous (pl. 23, fig. 16), and may be arranged more or less in lines corresponding to the peripheral parabasals. Some of the chondriosomes in this region are very closely applied to the parabasals, so that it seems possible that there is some relation between the two structures.

The chondriosomes vary a good deal in size and form, as observed in material fixed in Zirkle's and Champy's fluids (fig. A, 6). Among them are some granules ranging in diameter up to a micron or more. The majority are rod-formed, being straight or slightly curved rods about one to two or even up to three microns long. Many are constricted, and some have the appearance of two knobs connected by a filament. A few appear as long filaments, or even as chains of granules. In some material all of the chondriosomes are rounded granules, but this is probably due to poor fixation. In cases where the body of the flagellate has been ruptured in making the smear, the chondriosomes of the broken part are often rounded, while those in the less disturbed cytoplasm are of the typical rod form.

ANTERIOR ENDOPLASMIC GRANULES

Duboscq and Grassé state that, in the endoplasm of the prenuclear region of *T. chattoni*, chondriosomes of the typical form, as described in the preceding paragraph, are lacking. There are present, however, numerous spherules which react like chondriosomes but, unlike the typical chondriosomes, resist acetic acid. These they called anterior lipoidal granules, stating that they are doubtless of lipoid nature, and suggesting that they may be formed by the disintegration of parabasal bodies.

In the three species of *Trichonympha* described in this section of the report, there are granules in the endoplasm anterior to the nucleus which answer to the same specifications, though the species differ from one another in respect to the forms of the granules.

Numerous small irregular bodies in the anterior endoplasm of *Trichonympha campanula* are characteristic of this species (pl. 22, figs. 8, 13; pl. 23, fig. 17). While those in the anterior part of the cytosome are most conspicuous, there are, in the postnuclear endoplasm, bodies similar in size, form and staining reactions which probably belong in the same category. They are especially closely packed in the most anterior portion, just behind the rostral tube, and sometimes a few are present within the rostral tube.

These bodies stain as do the chondriosomes by some methods (Zirkle's fixative—iron-haematoxylin; Champy-Kull), but other methods also serve to demonstrate them. They are clearly visible, though usually unstained, after fixation in Schaudinn's fluid with or without acetic acid, and even after the vapor of glacial acetic acid. On some slides prepared by the Mallory method, the bodies took a

golden yellow color. In heavily stained iron-haematoxylin preparations after Schaudinn's fluid, some of the bodies, which resemble the rest in form and size, stain black, but they lose the stain quickly during the process of differentiation. They are highly refractive and clearly visible in living material. The writer has been unable to stain them with neutral red, brilliant cresyl blue, or Janus green B.

In form these bodies are diverse and irregular, some being small rounded granules, some larger masses, while many, perhaps most, are groups of smaller granules in circles, loops, plates, and other forms. Their composition of small, grouped granules was clearly visible in certain material treated by the Zirkle—iron-haematoxylin method (fig. A, 7), but it is not evident in ordinary preparations.

Anterior endoplasmic granules similar in staining reactions and distribution to those described above are present also in *T. collaris* (pl. 20, fig. 1; fig. B, 1). In this species, however, they differ from those of *T. campanula,* in that they are smaller, more regularly rounded granules which are less conspicuous. They also are especially abundant just posterior to the rostral tube. Among them are many small granules which stain readily and deeply with iron-haematoxylin, a type of granule not present in *T. campanula.* Some of the haematoxylin-staining granules are sometimes present within the rostral tube. These two species of *Trichonympha* can readily be distinguished from one another by the structure of the prenuclear endoplasm.

The anterior endoplasmic granules of *Trichonympha sphaerica* (pl. 26, fig. 31) are small, spherical granules very abundant in the limited prenuclear endoplasm, where they are relatively more numerous, for the given area, than those of the other two species, and are not limited to the peripheral region. They are more smoothly formed, compact, isolated granules than are those of *T. campanula* and *T. collaris,* resembling closely in form and distribution those of *T. chattoni* mentioned above. Among them are a few small granules which stain readily with iron-haematoxylin, but these are relatively much less numerous than are those of *T. collaris.*

POSTERIOR ENDOPLASMIC INCLUSIONS

Trichonympha sphaerica possesses in the posterior endoplasm a type of inclusion characteristic of the species (fig. B, 2; pl. 26, fig. 31). This consists of numerous vacuoles or spherules stainable with neutral red and cresyl blue, which apparently correspond to the vacuome as it is described in many other Protozoa. The same type of spherule is not present in *T. campanula* or *T. collaris.*

In living, unstained material the spherules are clear and not conspicuously refractive. With neutral red they quickly stain red and then, as the preparation stands, become a golden yellow. The same staining reaction occurs in the wood within and outside of the flagellates. The spherules are not always smooth of outline, and on the surface of many there are some neutral red staining granules. With brilliant cresyl blue the spherules stain blue, as does wood.

In permanent preparations the spherules usually are unstained, or at least not vividly stained. With Regaud's haematoxylin after Champy's fluid they become black when heavily stained, but in the process of destaining the black soon gives place to a yellow brown. They are yellow brown in Delafield preparations after Schaudinn's fluid, and similar but paler after iron-haematoxylin. When the flagellates are killed by placing in absolute alcohol in which 1 per cent Janus green B has been dissolved, the spherules, like the wood particles in the cytoplasm of *Trichonympha,* take the stain. But they have not been stained intravitally with Janus green in weak solution.

In size the structures which stain with neutral red vary greatly, ranging from about seven microns down to small granules. The smaller granules, which are numerous, are distributed evenly among the minute endoplasmic granules described above, from which they can be distinguished because of the fact that the latter do not stain with neutral red. The average size of the larger spherules varies in different cases, but those of all specimens on a single slide for the most part agree with one another. Thus it is probable that it is the environmental condition in the intestine of the host which determines the degree of development of the spherules in *T. sphaerica.* In some preparations the spherules are large, filling the endoplasm. The diameter of the largest of these may be seven microns, many measure five or six microns, and the average size is about four microns. In others spherules of the larger sizes are absent, the average being two and one-half microns, two, or even as little as one micron. The spherules were replaced altogether by small granules in one set of material, and consequently were not conspicuous as a distinctive feature of *T. sphaerica.*

The spherules are not homogeneous, as fluid-filled vacuoles would be. The interior contains some unevenly arranged clear spaces and some granules. In preparations fixed in the vapor of glacial acetic acid (pl. 27, fig. 35) each is surrounded by a smooth, well defined, rather heavy wall beneath which is a clear space, the interior substance

having shrunken toward the interior. This interior substance appears indistinctly vacuolated and granular.

Corresponding to the spherules of *Trichonympha sphaerica* are numerous small granules in *T. campanula* which stain with neutral red. These are distributed unevenly throughout the postnuclear endoplasm, and usually some are present in the middle zone of the ectoplasm. In the ectoplasm these are generally restricted to the posterior region of the flagellated zone, near the nucleus, but occasionally they are spread throughout this portion. They agree in size with the smallest neutral red staining granules of *T. sphaerica*, and no larger spherules are present. Sometimes, but by no means generally, a few larger rounded bodies occur; but these, which are usually granular and often irregular in outline, probably are ingested food bodies, or residues of digestion, rather than spherules like those of *T. sphaerica*.

In *T. collaris*, as in *T. campanula*, are many small, neutral red staining granules, but these are less abundant than in the other species, and the writer has not observed them to be present in the middle ectoplasmic zone, where they sometimes occur in *T. campanula*.

In its normal environment *Trichonympha* takes fragments of wood into the body. Sometimes the cytoplasm of the postnuclear region is packed full of fragments, most of which are small, but relatively large ones are often present. *T. sphaerica* usually contains less wood than the other two species. When the hosts are fed upon filter paper, *Trichonympha* ingests the particles of cellulose, but not in as large quantity as it ingests wood.

Besides the wood or cellulose, some individuals of *T. collaris* contain other organisms enclosed in vacuoles. Rod-formed bacteria, *Trichomonas*, *Streblomastix*, and *Tricercomitus* have been observed within the cytoplasm of this species. In one case more than seventy individuals of *Tricercomitus* were enclosed in one *Trichonympha collaris*. Bacteria and Protozoa are much less frequently encountered in *T. campanula* and *T. sphaerica*.

Another type of inclusion is often present in *T. collaris*. This consists of a variable number of bodies, which stain with neutral red and cresyl blue, like the spherules of the vacuome, but, unlike the latter, readily stain also with Delafield's and iron-haematoxylin. They are generally numerous in *T. collaris* from wood-fed termites, and are seldom, if at all, present in *T. campanula* or *T. sphaerica*. The bodies are often unevenly spherical, often irregular in form, sometimes elongated (pl. 25, fig. 28). Their size varies greatly, some being 12μ

in diameter, some very small. On the surface and throughout the mass are often granules, flecks, or rodlets which stain deeply. Each is enclosed in a vacuole, which possibly indicates that they are residues of digested food, or material in the process of digestion. Probably this is not wood, for *T. sphaerica* and *T. campanula* contain wood, but usually do not contain these bodies. Possibly they are the remains of the other organisms which *T. collaris* often ingests.

COMPARISON OF THE THREE SPECIES IN TERMOPSIS

With the above characteristics in mind, aided by a comparison of the figures (fig. B), one should encounter no difficulty in identifying *T. campanula, T. collaris,* and *T. sphaerica.* The differences are clear-cut, there is no intergradation in most of the criteria used, and there consequently is no question of the species being based merely on stages in the development of one organism.

T. sphaerica can easily be distinguished from the others by its smaller size, with the shorter rostrum, and proportionately greater breadth of rostrum and body; by the anterior situation of the nucleus; by the relatively narrow and short cone of prenuclear endoplasm, with the abrupt shoulder at its base; by the absence of distinct zonation of the flagella; and by the neutral red staining spherules in the postnuclear endoplasm, which are not present in the other two species, though represented in them by neutral red staining granules

T. collaris can be distinguished from *T. campanula* by its slightly larger size and generally stouter build; by the presence of peripheral granules, which can be easily seen in living material, in the outer layer of ectoplasm; by the arrangement of parabasal cords in groups passing close to the nuclear membrane and extending farther into the postnuclear endoplasm than do the slender, isolated cords of *T. campanula;* by the larger size and more anterior situation of the nucleus; and by the absence of the "heterochromosome" in the nucleus.

In the writer's experience, the three species are present in comparable numbers, often about equal, instead of *T. sphaerica* being as rare as reported by Cleveland (1925*b*) and Lund (1930).

The protozoan faunas of *Termopsis angusticollis* and *T. nevadensis* are identical. The list comprises seven flagellates and a gregarine. The gregarine has been found in a small percentage of colonies only. The writer formerly mentioned their occurrence in *Termopsis* (1927), and has since observed them in one colony each of *Termopsis angusti-*

collis and *T. nevadensis,* both from Carmel, California. Besides the three species of *Trichonympha,* there are *Trichomonas termopsidis* Cleveland, *Streblomastix strix* Kofoid and Swezy, *Tricercomitus termopsidis* Kirby, and *Hexamastix termopsidis* Kirby.

Fig. B. Diagrams of species of *Trichonympha* from *Termopsis.* × 250. Sizes and forms typical, former derived from averages. 1. *T. collaris* sp. nov. 2. *T. sphaerica* (Kofoid and Swezy). 3. *T. campanula* Kofoid and Swezy.

In *Termopsis laticeps* there are five flagellates: *Trichonympha campanula, Trichomonas termopsidis, Streblomastix strix, Tricercomitus termopsidis,* and *Hexamastix laticeps.* Thus *T. laticeps* differs from the others in possessing only one species of *Trichonympha* and in having a markedly different species of *Hexamastix.* These differences in the faunas are correlated with the fact that *Termopsis laticeps,* which is isolated in distribution from the other two species, is morphologically distinct from them in much greater degree than they are distinct from each other.

ENTOZOIC MICROORGANISMS

Six microorganisms have been found living in the bodies of the species of *Trichonympha* in *Termopsis.* This number includes *Sphaerita,* which has been reported from *Trichonympha* by previous authors; the others have not been described from any Protozoa of termites.

At first sight, it may seem difficult to determine whether a microorganism is truly entozoic, living and multiplying in the cytoplasm of its host, or is but an object ingested by the flagellate for food or together with the food. It is probable, however, that in the case of the microorganisms described below, this error was not made. From a consideration of their occurrence, distribution, staining reactions, and the stages in their life-histories, it seems likely that they are true parasites, which live in the cytoplasm from which, presumably, they derive nutriment.

A survey of the literature on the parasites of the Protozoa has revealed no organisms similar to any of the parasites of *Trichonympha,* except *Sphaerita,* so descriptions of them are presented below without determination of their systematic status.

PROXIMO-NUCLEAR PARASITE

Plate 22, figure 8; plate 23, figures 14, 15, 18, 19; plate 31, figures 73–76

When one observes stained specimens of *Trichonympha campanula* or *T. collaris,* one of the most conspicuous features of suitably prepared material is the existence of what appears to be a mass of small granules surrounding or situated in the vicinity of the nucleus. Careful observation shows that, although some of these apparently are granules, most are rods or filaments of great diversification in length. They vary in abundance in different flagellates, but have been present in all of hundreds of specimens of these two species of *Trichonympha* which the writer has observed. They are not present in *Trichonympha sphaerica.*

The parasites usually are aggregated into a dense mass in *T. campanula;* into a more diffuse mass, spread across the endoplasm at the level of the nucleus, in *T. collaris.* In *T. campanula,* the mass sometimes surrounds the nucleus and is densely packed (pl. 23, fig. 14; pl. 31, figs. 73–74); but often it is in the endoplasm anterior to or just behind the nucleus and less densely packed (pl. 22, fig. 8: pl. 23, fig. 15; pl. 31, figs. 75–76). Of 130 specimens of *T. campanula* on one slide from *Termopsis laticeps,* just half had the group surrounding the nucleus, and half had it in the prenuclear endoplasm.

The staining reactions of these bodies are those of microorganisms. They stain intensely with iron-haematoxylin and Delafield's haematoxylin, after all the fixatives used, and retain the stain more tenaciously than any structures of *Trichonympha,* including the chromatin.

In material prepared by Feulgen's method followed by light green, the mass took a purple color.

Around the edges of the mass it is possible to study the structure of the microorganisms. Most of them are slender filaments or rods of diverse lengths, but usually rather short, and many are bent or curved in various ways. Those shown in the figure (pl. 23, fig. 19) are from four to eight microns long; this is a frequent size, but sometimes much longer ones are encountered. The rods, which are blunt at the ends, contain a number of relatively large granules, which occupy the entire width of the body. These granules stain intensely with haematoxylin, and aid in giving to the group the appearance of a dense aggregation of granules.

This parasite, unlike any others observed in *Trichonympha*, appears to occur constantly in the endoplasm of *Trichonympha campanula* and *T. collaris*. The fact that it is almost always very close to, and often, as a group, surrounds the nucleus, may indicate that it depends for its nutrition upon immediate proximity to the source of nuclear influences upon metabolism.

SMALL CLUSTERED GRANULES

Plate 23, figure 14; plate 24, figure 20; plate 31, figures 73–74

Irregular clusters of small granules have been observed in a large percentage of the specimens of *Trichonympha campanula* from *Termopsis laticeps*, but not in *Trichonympha* from the other two species of *Termopsis*. Of 121 individuals on one slide, this parasite was present in 48. When the group of proximo-nuclear parasites surrounds the nucleus, these clusters are often gathered just posterior to the group (pl. 23, fig. 14; pl. 31, figs. 73–74). In other cases the clustered granules are dispersed throughout the endoplasm, chiefly in that part posterior to the nucleus (pl. 24, fig. 20). Some are single, isolated granules, but most are collected into clusters usually consisting of a considerable number and very irregular in form.

The granules stain deeply with Delafield's haematoxylin after fixation in Schaudinn's fluid.

Their minute size, as well as the irregularity in form of and great variability in number composing the clusters, distinguishes them readily from *Sphaerita*. They resemble the irregular groups of granular bacteria described by Sassuchin (1928) from *Nyctotherus ovalis*. In *Trichonympha*, however, no such very large aggregates of granules as some of those described in the ciliate were observed.

PEG-FORMED PARASITE

Plate 24, figures 21–24

A small, peg-formed parasite has been found occasionally in *Trichonympha campanula* from *Termopsis angusticollis* and *T. nevadensis*, but not from *Termopsis laticeps*, nor in the other two species of *Trichonympha*. It was present in preparations from a number of termites of at least two colonies, but in all cases had a very low incidence, not more than one out of three or four hundred flagellates being infected. On ten slides of one series only four parasitized flagellates were encountered, two on each of two slides from different termites.

The endoplasm of the postnuclear portion of the infected flagellates contained a large number of the parasites, which were quite uniformly distributed. In some cases, but not in most, a number of them were present in the region around the nucleus and in the anterior portion (pl. 24, fig. 21).

The parasites are of quite uniform size, in length usually ranging from two to three and one-half microns, in diameter at the broad end from one-half to three-quarters of a micron. They are peg-shaped, being broadly rounded at one end, and tapering toward the other end, which is pointed (pl. 24, fig. 22). At the broad end there is almost always a clear area, occupying about a quarter to a third of the length of the body. The remainder of the substance stains deeply with iron-haematoxylin, and becomes dark olive green in material prepared by the Champy-Kull method. The clear area was not stained by any of the methods used.

Associated with these peg-formed bodies, in some cases, there were longer, rod-like structures, up to about twelve microns in length, and of diameter about the same as that of the others. These stain more lightly than the others by all the methods used, and show no clear areas. Many of them are constricted in the middle (pl. 24, fig. 23).

In two cases there was found another form of parasite which probably also is assignable to the life-history of this species. In the flagellates containing this form longer rods like those described in the preceding paragraph were present, but there also were chains of bodies from two to five microns long, which stained uniformly and intensely, seldom showing any indication of the clear area at one end (pl. 24, fig. 24). Associated with these were some peg-formed bodies of the usual form, but without the clear area.

Possibly the parasite grows into the long rods, which divide up by constriction into the peg-formed bodies. In the latter form, in the material examined, the parasite was most frequently encountered.

FUSIFORM PARASITE

Plate 25, figures 25–27

A parasite which when fully grown has a spindle form was found fairly frequently in *Trichonympha campanula* from *Termopsis laticeps*, but not in the flagellates from the other species of termites. Its incidence was higher than that of the peg-formed parasite, as it was present in 10 of 116 individuals on one slide, and on other slides in comparable abundance. The parasites were usually restricted in their distribution to the region posterior to the nucleus, but sometimes a few occurred in the prenuclear region.

Most of the parasitized *Trichonympha* contained a number of spindle-formed bodies, twelve to twenty microns long and one and one-half to three microns broad across the middle, which tapered to points, and were often drawn out a short distance at the ends (pl. 25, fig. 25). Each of them appeared to be surrounded by a delicate membrane, between which and the stainable body of the parasite was a clear space (pl. 25, fig. 26). The substance of the parasite stained deeply with Delafield's haematoxylin, except, frequently, for a small portion at one or both ends. Within its body no differentiated structures were observed.

In a few flagellates there occurred, in addition to the larger stages of the parasite, smaller forms ranging down to short, slender rods (pl. 25, fig. 27). A complete series was found between the smallest rods and the larger, fusiform bodies, so it is probable that these all represent stages in the life-history of the microorganism.

SPHAERITA

Plate 27, figures 36 and 37

This parasite, unlike any of those previously described in this paper, has been encountered frequently in a wide variety of Protozoa living in termites, as well as in other Protozoa. Its incidence, however, was not high in the species of *Trichonympha* from the termites of the genus *Termopsis* which were examined. It has been found most frequently in *Trichonympha sphaerica* (pl. 27, figs. 36, 37), which was not infected by any of the other entozoic microorganisms, but it occurred also in *T. campanula* and *T. collaris*.

MOTILE INTRACYTOPLASMIC MICROORGANISM

An unusually interesting parasite has been observed in *Tricho-nympha collaris* and, rarely, in *T. campanula* from one colony of *Termopsis angusticollis*. The incidence was not high, nor was the parasite found in flagellates from all termites of the colony. In a survey made to determine the incidence, a small number of flagellates in only two of seven termites of the colony were found to be infected.

The parasites may be detected readily in the prenuclear endoplasm by the clear tracks, which are constantly changing in form, left in the wake of the moving bodies. The organisms are ellipsoidal or ovoidal, with clearly defined margins. Their size varies greatly; the smallest one observed was not more than one micron in diameter, while the largest in the same flagellate measured fifteen microns. The maximum size among those measured was twenty-one microns, but most were not more than five microns in length. They are sometimes numerous in a flagellate; in one case more than thirty, of various sizes, were present. Though most easily seen in the prenuclear endoplasm, they may also be present in the postnuclear endoplasm, and one large active one was enclosed within a nucleus.

Some of the parasites are perfectly clear, with no visible granular contents. In many there are some minute granules, small bits of wood and sometimes also bacilliform bodies. These are in some cases in movement within the parasite, indicating the fluidity of its protoplasm.

The parasites usually move steadily and fairly rapidly through the cytoplasm, traveling in different directions and in straight or sinuous courses. Their speed, while varying, is not in ratio to their size; small ones may advance as rapidly as large ones. As the parasite progresses it spreads apart the densely packed minute endoplasmic granules, and, as these do not close in at once, a clear wake is left behind. The length of the wake, of course, varies with the speed of progression, in most cases being four to ten times the length of the parasite.

There are movements of the body of the organism itself which are amoeboid in that the outline changes, but typical pseudopodia are not formed and many move forward with very little change of shape. When granules are present in the deeper endoplasm of the parasite they have an irregular dancing movement, and there is no indication of cytoplasmic streaming. At times, in locomotion, the posterior part of the body is drawn out.

As to the nature of this organism, the writer can at present offer no suggestion. Its independent, relatively rapid movement shows clearly that it is an active microorganism of some kind. But no nucleus or locomotor organs have been seen, as it has not been possible to prepare the parasite in a manner suitable for demonstration of these.

TRICHONYMPHA IN HODOTERMES

The termites of the subfamily Hodotermitinae contain faunas of flagellates some members of which are related to flagellates which occur in other subfamilies of the Kalotermitidae, while others are related to some of those of the Rhinotermitidae. Two species have been thoroughly investigated: *Hodotermes* (*Hodotermes*) *mossambicus* of South Africa, which does not contain *Trichonympha* (Dogiel), and *Hodotermes* (*Anacanthotermes*) *murgabicus* of Turkestan, which contains *Trichonympha turkestanica* (Bernstein).

The fauna of the former species differs markedly from that of the latter. Since, as reported below, the fauna of another species of the subgenus *Anacanthotermes* has been found to agree in general with that of *H.* (*A.*) *murgabicus*, it may be that there is a general cleavage in flagellate infection between the two subgenera.

From *H. mossambicus* Dogiel (1916, 1917a, 1922) reported flagellates of the genera *Trichomonas, Gigantomonas, Myxomonas* (which is a synonym of *Gigantomonas*), *Joenia, Holomastigotes* and *Spirotrichonympha*. Associated with *Trichonympha turkestanica* in *H. murgabicus*, Bernstein (1928) reported *Holomastigotoides cingulatum, Holomastigotes magnum, Microspironympha porteri, Stephanonympha dogieli, Trichomonas vermiformis, Eutrichomastix termitis*, and *Devescovina elongata*.

In *Hodotermes* (*Anacanthotermes*) *macrocephalus* of India, of which specimens preserved in alcohol were given to the writer by Dr. S. F. Light, there occurs a species of *Trichonympha* corresponding to *T. turkestanica* in all characters observable in such material. The remaining fauna appears also to be similar to that recorded by Bernstein, the writer having observed forms which resemble *Holomastigotoides, Microspironympha, Holomastigotes*, and the so-called *Stephanonympha*. All flagellates except *Trichonympha* were poorly preserved.

De Mello (1920a, c) gave some attention to the fauna of *Hodotermes viarum* (Koenig) from Coimbatore, India, preparations of

which had been sent to him by Mr. Bainbridge Fletcher. The species of termite is not identifiable, according to Snyder. From its distribution it seems possible that it was *Hodotermes (Anacanthotermes) macrocephalus*. De Mello reports having identified *Trichonympha agilis* Leidy, *Leidya metchnikowi* França, and *Treponema termitis* Leidy and (1920c) states that numerous other species are also present. His figures represent flagellates, one of which is doubtless *Trichonympha* and the other possibly *Holomastigotes*. The former, however, is certainly not *T. agilis*. Two of the figures of *Trichonympha* represent animals measuring, according to the accompanying scale of microns, 110 and 170μ in length. While considerably larger than *T. agilis*, these fall within the range of *T. turkestanica*. De Mello's species may have been similar to, if not identical with, that species.

A brief account of two flagellates from a termite of Calcutta, India, was published by Simmons (1890), but unfortunately his drawings were not reproduced. One of the flagellates, which, he states, averaged 200 × 125μ in size, was probably either *Trichonympha* or *Pseudotrichonympha*; the other was probably one of the Spirotrichonymphidae or Holomastigotidae. There is a resemblance between the forms described and figured by De Mello and those described by Simmons. It is possible that they observed the same species of flagellates. Incidentally, Simmons was apparently the first to note that certain termites are not infected with Protozoa. He studied some "Behar termites" which had been sent to him, and found no Protozoa in those he examined.

Trichonympha turkestanica Bernstein, 1928

Hosts: *Hodotermes (Anacanthotermes) murgabicus* Vasiljev. Turkestan.
Hodotermes (Anacanthotermes) macrocephalus Desneux. India.

Trichonympha turkestanica is as long as the smaller specimens of *T. campanula*, ranging in length, according to Bernstein, from 140 to 250μ, and in the writer's specimens from 120 to 264μ. Bernstein gives the width as ranging from 43 to 115μ, averaging 72μ, which may be compared with the average width of 85μ for *T. campanula*. In the writer's material, however, the organisms were much more slender, from 50 to 60μ; this was probably the result of poor preservation. In the form from *H. (Anacanthotermes) macrocephalus*, the measurements of the rostrum were: length in center, from 13 to 15μ; diameter at base of cap, 11μ; diameter at base of collar, from 22 to 24μ. Bernstein does not give the rostral measurements, or the magnification of

the figures, but the proportionate size of the rostrum in her drawings appears to be the same.

The layers of ectoplasm are, contrary to the description by Bernstein, similar to those observed in other species of *Trichonympha*. In the rostrum the dense inner and clear middle layers, which can easily be distinguished even in the alcoholic material, are shown by her combined as the undifferentiated wall of the tube. Outside of the slender, refractive rostral tube the inner and middle layers are of about equal thickness, and the outer layer is thicker than both combined. In the body region there also are three layers of ectoplasm, the outer one containing the plates, the middle one (inner layer of Bernstein) striated by the roots of the flagella, and the inner one (not described by Bernstein, but shown by her in text fig. 2), formed much like that of *T. collaris*.

At the anterior end of the rostral tube is a hemispherical blepharoplast, similar to but relatively larger than that of *T. collaris*. It is not so large as the hemispherical structure shown within the cap by Bernstein, but outside it, within the cap, is a membrane, or at least a clear zone with a definite border, which corresponds in size to that structure. The dense blepharoplast apparently was overlooked by Bernstein.

Within the anterior part of the rostral tube, extending for about half the length, is a rod formed by constriction of the endoplasmic core, which posteriorly broadens to the diameter of the inside of the tube. There is a similar rod-like core in *T. collaris*, but this occupies only about a sixth of the length of the tube. Between the rod and the walls of the anterior portion of the tube is a clear space.

The stainable, ring-formed structure posterior to the fissure, which Bernstein calls the centroblepharoplast, is probably no more than the denser ectoplasm present in this position in all species of *Trichonympha*. In some iron-haematoxylin preparations this region of the body stains heavily, and it is impossible to make out its true structure.

Bernstein states that the number of plates is from 95 to 98, which is about the same as the number in *T. campanula* and *T. collaris* (97–112). Between the plates her figures show small granules, which are similar in form and distribution to the peripheral granules of *T. collaris*. She calls these basal granules, but they are not in the usual position of basal granules, nor are they regularly arranged. It has not been possible for the writer to distinguish the peripheral granules in the alcoholic material from *Hodotermes macrocephalus*.

The flagella on the rostrum are not longer than those on the anterior portion of the body, as they are in *T. campanula* and *T. collaris*. The long posterior flagella extend beyond the end of the body.

In the prenuclear endoplasm are many small granules, which were very distinct in the alcoholic material.

The nucleus is near the middle of the body, and the proportion between flagellated and non-flagellated areas is similar to that in *T. campanula*.

Bernstein does not report the parabasal apparatus, which she designates as the suspensorial apparatus of the nucleus, and it has not been possible for the writer to detect any trace of it in the alcoholic material. It is improbable, however, that this is absent, although Bernstein states in the key to the species of *Trichonympha* that it is absent in *T. turkestanica*.

TRICHONYMPHA IN POROTERMES

Trichonympha magna Grassi, 1917

Hosts: *Porotermes adamsoni* (Froggatt). Australia.
　Porotermes grandis Holmgren. Australia.

Plate 28, figures 43–48; plate 30, figure 66; plate 31, figure 78

Grassi's account gives no very specific characteristics for *Trichonympha magna* from *Porotermes adamsoni*, which would serve to distinguish it from other closely related species. It is probable, nevertheless, that the *Trichonympha* found in abundance in *Porotermes grandis* belongs to the same species; especially since, as pointed out by quotation from Hill (Kirby, 1931) the two forms of termites may belong to the same species. *Pseudotrypanosoma giganteum*, which Grassi described from *P. adamsoni*, has been redescribed by the writer (1931) from *Porotermes grandis*. Flagellates of this species with the dimensions recorded by Grassi have since been encountered in alcoholic material. The other members of the fauna in *P. adamsoni*, as described by Grassi, and in *P. grandis* are: *Joenina pulchella*, *Spirotrichonympha mirabilis*, and *Spirotrichonymphella pudibunda*.

Trichonympha magna has been studied in material sent by Mr. G. F. Hill from Fern Tree Gulley, Victoria, Australia. The preparations consist of a few whole mounts and some sections fixed in Schaudinn's fluid and stained in Delafield's or iron-haematoxylin, in the latter case with counterstain of acid fuchsin.

In the writer's material the length ranged from 114 to 172μ, the width from 42 to 60μ. The rostrum, similar in structure to those of the species from *Termopsis*, measures in length in the middle from 12 to 13μ, in diameter at the base of the cap from 6 to 8μ, and in diameter at the base of the collar from 16 to 20μ. The flagellated region is extensive in this species, as compared with *T. sphaerica* and the forms from *Kalotermes*, occupying from half to two-thirds of the length of the body (pl. 28, fig. 43).

The slender rostral tube extends a few microns beyond the posterior end of the rostrum before it merges into the layer of basal granules. The blepharoplast is a hemispherical granule smoothly convex anteriorly, its margins corresponding in position to the outer boundary of the inner layer of ectoplasm. It stains intensely with iron-haematoxylin, but loses the stain readily, after which it may be red from the counterstain. At its base, where it is met by the rostral tube, there is a more deeply staining portion, where two deeply staining granules appear in optical section. From the blepharoplast the anterior margins of the outer layer of ectoplasm slope posteriorly quite steeply, forming in section an angle of about ninety degrees. The cap covers this region; usually it is partly collapsed in fixed material.

In the absence of fresh material or of whole mounts very satisfactory for the purpose, it has not been possible to measure the flagella, but it is clear that the arrangement is similar to that in *T. campanula* and *T. collaris*. The rostrum bears long flagella, while on the body region are shorter ones save near the posterior part of the flagellated region, where arise long ones clothing the posterior portion of the body and extending beyond it.

Near the posterior end of the flagellated zone the ectoplasm is about eleven or twelve microns thick. The middle layer is especially well developed, being relatively thicker than in any other species studied. Instead of decreasing in thickness posteriorly, as in many species of *Trichonympha*, its thickness increases from its anterior to its posterior end, where it terminates abruptly. Here its thickness is about five microns. The outer layer of ectoplasm is much thicker than the middle layer in the rostrum and the anterior part of the body region, but in the posterior portion of the flagellated zone it is about equal to it, or sometimes even thinner. From the level of termination of the middle layer it extends backward, tapering outward toward the surface, so that the posterior point of origin of flagella from the surface of the body is some distance posterior to the basal granules

from which these flagella arise. The inner layer, which is much like that of *T. collaris*, is thin and tapers posteriorly.

The plates in the outer layer, like those in *T. collaris*, are moderately thick (pl. 28, fig. 44). The number of plates in the body region was counted in five specimens; three had 40, one 42, and the other 46. The surface between the plates is raised into rounded ridges. The roots of the flagella extending through the middle layer pass through the plates. The outer halves or less of the parts in the middle layer are thicker than the rest. Where the stout and slender portions meet, the former is deeply staining and sometimes apparently slightly enlarged, so that there may appear to be a granule here (pl. 28, figs. 44–45). That, however, is not actually the case.

The middle and outer layers are sharply separated; between the bases of the plates is protoplasm which stains red with the counterstain. There is a boundary here, which sometimes appears in whole mounts as a line, but it is not a membrane or a layer of granules. The inner layer is dense in appearance, staining red with the counterstain. In the rostrum it is thicker than the middle layer, in the anterior body region as thick as that layer, but it thins out posteriorly. The basal granules lie in its innermost portion. In the posterior part, where this layer thins out, there appears a well defined line between it and the middle layer. The line may be stained while the region on both sides of it is clear. The roots of the flagella pass through it. This stainable boundary appears to be continuous, not a layer of fibers.

The basal granules, which have been satisfactorily studied only in sections, are small granules, instead of rodlets as in the case of *T. campanula*. The rostral tube is solid to the point a short distance posterior to the base of the rostrum where it begins to spread. Beyond this point, for a distance somewhat less than the length of the rostrum, the basal granules are comparatively large, deeply staining, and conspicuous in sections. More posteriorly they are smaller and not so conspicuous. In the anterior region they are arranged in transverse rings which are about half a micron apart. They are much closer together transversely than longitudinally; in the more anterior portion the rings appear continuous even in transverse sections, separate granules not being distinguishable. More posteriorly, however, they may be resolved into separate granules, situated close together. In haematoxylin-stained material the endoplasm of the subrostral region appears covered by from 10 to 12 black rings. It has not been possible to distinguish any longitudinal connections between these basal gran-

ules, nor any transverse connections apart from the close proximity of the individual granules.

The parabasal apparatus (pl. 28, fig. 43) consists of numerous cords, the number of which has been estimated in a few cases to be from about 40 to 45. The individual cords are round in section and somewhat variable in thickness, but many are about one micron thick. They are smooth and have stainable borders, indicating the existence of a sheath. In some cases one border appears much heavier than the other; doubtless this is the chromophile part or parabasal thread. The sheath and thread are black in some iron-haematoxylin—acid fuchsin preparations, while the substance within is red from the counterstain.

The anterior portions of the parabasal bodies lie in the peripheral endoplasm close to the posterior end of the middle and inner layers of ectoplasm. Here they end, perhaps being attached to the layer of basal granules; they do not extend anteriorly. It is possible that the parabasal cords correspond in number with the plates, or the rows of basal granules. From their point of origin they extend back toward the nucleus, meeting the membrane at a point near or anterior to the middle. Then they extend along the membrane in a single layer, leaving it near the posterior end to continue for a short distance into the postnuclear endoplasm. Posterior to the nucleus they are very sinuous or coiled, and are irregularly grouped.

The separate parabasal cords around and anterior to the nucleus lie in a single layer close to one another (pl. 28, fig. 48). The margins of two adjacent cords are, in cross-section, distinct and separate, but it is possible that there is some intervening attaching substance which causes them to remain parallel to one another as they do.

From the study of sections it appears clear that while the parabasals lie adjacent to the nuclear membrane they are not attached to it. Where the membrane is collapsed it draws away from the parabasals, and in cross-section there is a narrow clear space between the layer and the membrane, perhaps owing to shrinkage of the nucleus.

The nucleus (pl. 30, fig. 66; pl. 28, fig. 48) is situated close to the limits of the flagellated region, thus being near or posterior to the middle of the body. Usually the posterior limit of the nucleus is a few microns (10–15) beyond the ends of the plates. Its diameter ranges from 13 to 20μ, averaging about 17μ. In no case has the chromatin been found to be dispersed in separate granules, and there is no evidence of a linin reticulum. The chromatin is organized into

more or less varicose strands, coiled and twisted, and interconnected in places by chromatic filaments. In most cases the strands are distributed throughout the nucleus, often extending to the membrane, and in other cases connected by a few filaments to the membrane. In a few cases, however, they are packed together into a central chromatin mass, leaving a clear space of relatively considerable extent between it and the membrane. At one point near the periphery is a spheroidal nucleolus (pl. 30, fig. 66) which in the material examined was not stained with iron-haematoxylin, but took the acid fuchsin counterstain.

The prenuclear endoplasm does not contain a group of granules occupying most of the space enclosed by the ectoplasm, as in *T. sphaerica* and other species to be described below. Rather does it appear quite clear in section, save for scattered, minute granules. Down the center, however, from the rostrum to the nucleus, is a structure which occurs in this species alone of those described in this paper. This is a cylindrical column, variable in its thickness but often two or three microns, extending from behind the base of the rostral tube toward the nucleus (pl. 28, fig. 43). Often it reaches this, sometimes it stops short of it. Its outlines are uneven, its course not straight, and it does not possess any enclosing sheath. Surrounding it, in the fixed material, there is always a clear space, which is somewhat variable in extent. The column took the counterstain in iron-haematoxylin —acid fuchsin stained material. Under ordinary magnification it appears homogeneous, but, as disclosed by critical observation, it seems to consist of minute granules, closely packed together. Thus, possibly, it corresponds to the groups of granules in other species.

This finely granular column has been observed in almost all specimens examined. Though Grassi gives no indication of its presence, it is a very characteristic feature of the species. In a few cases there is a large, stainable mass in the endoplasm posterior to the rostrum. It is broad and variable in extent, in one specimen filling almost all the prenuclear area. When this is present, the column is absent or poorly developed. Possibly the stainable mass represents an abnormal or unusual development of the same material which goes to form the column, as it seems to be similar in texture.

The postnuclear endoplasm contains wood and many small granules but, as in *T. campanula* and *T. collaris*, there are no spherules like those of *T. sphaerica* and all species from *Kalotermes* described below.

In each of a number of flagellates there was present a large vacuole from 25 to 30 microns in diameter, containing many individuals of a certain microorganism in various stages of development. The infected flagellates were for the most part grouped together in certain regions of the intestine. No microorganisms of the same kind were observed outside of the bodies of *Trichonympha,* except where some had been dragged out in cutting the section. The large, spherical vacuole possessed a well defined boundary but no heavy membrane, and the contents consisted entirely of the microorganisms here described.

The smallest stages figured (pl. 28, figs. 46–47) are minute, elongate ellipsoidal bodies about two microns long, containing a number of haematoxylin-staining granules. Forms of the same type up to five microns in length are present; some of the larger ones are constricted in the middle or partly divided into two equal bodies like those first described.

Together with them and much more abundant are larger bodies from about five to nine microns long. In the smaller of these, which are similar in stainability and granular contents to those first described, there is a concentration of substance at one end. This concentration consists of a denser grouping of granules surrounded by a stainable, possibly granular boundary, forming a sphere occupying about half the length of the cell and the full diameter of the larger end. The diameter of this end increases to about four microns in cells nine microns long. In what appear to be early, but not the earliest, stages the globular vesicle contains small granules and a larger, spherical body which stains red with acid fuchsin. This last appears to grow larger in size until it almost completely fills the vesicle, when its staining capacity is lost.

In the largest cells, consequently, one end encloses a spherical vesicle which is quite clear. The wall of this stains heavily in places, however, especially in the part where it meets the cytoplasm. Within the vesicle is a slightly grayish body, between which and the wall is a clear space. Extending down the center of the cell, beginning at the vesicle wall and reaching about halfway to the end, is a relatively stout line which stains black with iron-haematoxylin. On the sides of the posterior part are stainable bands, indicating a peripheral accumulation of chromatic material. In no part of the cell are any chromatic granules like those which exist in smaller stages. The internal substance of this narrower portion of the cell stains gray, and is often

clearer near the vesicle. In a few exceptional cases the contents of the vesicle did stain rather deeply with iron-haematoxylin.

Possibly this microorganism is a bacterium which divides transversely in stages which are small, and which usually, in the material studied at least, enlarges and forms a spore in one end of the cell.

TRICHONYMPHA IN KALOTERMES *sensu latu*

Trichonympha chattoni Duboscq and Grassé, 1927

Hosts: *Kalotermes* (*Glyptotermes*) *iridipennis* Froggatt. Australia.
Kalotermes (*Kalotermes*) *contracticornis* Snyder. Costa Rica.
Plate 22, figure 10, parabasals; plate 30, figures 59–60, nuclei

This, the first species to be reported from a termite of the genus *Kalotermes*, *K.* (*Glyptotermes*) *iridipennis*, has been described in detail by Duboscq and Grassé (1927*a* and *b*). A hypermastigote flagellate which, agreeing with it in every way, must be assigned to the same species, occurs in *K.* (*Kalotermes*) *contracticornis*. Although, so far as can be determined from morphology alone, the forms of *Trichonympha* in the two termites belong to the same species, there is a considerable difference in the faunas as a whole. In *K. contracticornis* there are at least three genera of devescovinids, one of which resembles *Gigantomonas;* while in *K. iridipennis* the only devescovinid reported is *Devescovina hilli*. The former species contains *Trichomonas cartagoensis* Kirby, 1931, while from the latter no species of *Trichomonas* has been reported.

Duboscq and Grassé give the length of *T. chattoni* as from 60 to 125μ, though in another connection they mention forms of 180μ, which, however, possibly had undergone an abnormal elongation caused by the manipulations of smearing. The specimens measured from *K. contracticornis* ranged from 84 to 132$\mu \times$ 36 to 57μ. The anterior flagellated region occupies about a third, more or less, of the body length. The original authors state that the anterior flagellated part occupies only about a sixth of the length, but their figures do not bear this out. The rostrum measures in length about 12μ, in diameter at the base of the cap about 6μ, and in diameter at the base of the collar about 13μ.

In this species there is a sharp line of demarcation between the outer and middle layers of ectoplasm. This takes the form of a stainable line; whether continuous or a line of granules could not be

decided. The middle layer is very narrow in proportion to the outer layer; in the rostrum it is not so wide, and in the body region is no wider than the dense inner layer. The middle and inner layers are not clearly separated from one another, and in many haematoxylin preparations cannot be separately distinguished. The error made by Duboscq and Grassé of showing a thick, dense inner layer which really comprises the inner and middle layers is an easy one to make in this species, in the absence of comparative material of other species.

The blepharoplast, at the anterior end of the rostral tube, is a flattened granule broadly convex anteriorly, flat or shallowly concave posteriorly, and the haematoxylin-stainable material is sharply downcurved at the margins where it is apparently continuous with the outer border of the inner layer of ectoplasm. While in many preparations the blepharoplast appears as a single granule, in many others two smaller granules appear at the top of the rostral tube, just as described by Duboscq and Grassé (1927b, pl. 17, fig. 23). These seem to be actually separate granules, not sections of a ring. Covering them both is the rest of the blepharoplast substance, which Duboscq and Grassé do not report. The blepharoplast of *T. chattoni*, then, is a hemispherical mass which may stain with haematoxylin deeply as a whole, but when further destained discloses two especially stainable granules within its basal portion. With further differentiation still, these lose their blackness and the entire blepharoplast appears brownish or yellowish in iron-haematoxylin preparations.

The parabasal apparatus has been studied in material stained in Delafield's haematoxylin after Schaudinn's fluid, in which it is rather obscure. The cords agree in size and arrangement with those described by Duboscq and Grassé. They are usually fairly straight, and most are free from contact with the nucleus, though some may touch it. In this material the chromophile filament was well stained, while adjacent to it the chromophobe substance, though pale, showed indications of the vacuolization described by Duboscq and Grassé (pl. 22, fig. 10). According to the writer's observations, which in this point differ from those of the French authors, the chromophobe substance is surrounded by a delicate sheath which stains with haematoxylin, so that the cords do not have the uneven outlines indicated in their figures.

The nucleus, which ranges in diameter from 6 to 13μ, averaging from 10 to 11μ, is constructed as described by Duboscq and Grassé, with chromatin in granules, rods, threads, or masses filling most of

the interior, and with a spheroidal nucleolus of moderate size located in a vesicle near the periphery (pl. 30, figs. 59–60). Early prophase nuclei have exactly the appearance shown in their figures. It has been possible, also, to verify their observation that the paradesmose does not connect the bases of the rostral tubes.

In the postnuclear endoplasm of this species, as in *T. sphaerica* and most of the other species from *Kalotermes* described below, are numerous spherules of variable size, from about one to five microns in diameter. These are vacuolated and granular in the interior, and appear yellow brown in iron-haematoxylin preparations. In the absence of fresh material it has not been possible to determine whether or not these stain with neutral red, but their similarity to those which do so stain in *T. sphaerica* makes it probable that they do. Duboscq and Grassé record similar spherules, from two to three microns in diameter, from their specimens of *T. chattoni*. Besides these, the endoplasm sometimes contains large, irregular haematoxylin-staining spherules, like those shown by the French authors, as well as ingested wood and organisms.

In some specimens heavily stained with iron-haematoxylin after fixation in Schaudinn's fluid, there are deeply stained bodies which correspond in every way with the so-called mitochondria of *T. chattoni* from *K. (G.) iridipennis*. In many specimens, however, these are few in number or absent; it seems likely, therefore, that they have in some cases persisted despite the action of the acetic acid.

Trichonympha tabogae sp. nov.

Host: *Kalotermes (Kalotermes) tabogae* Snyder. Panama.

Plate 30, figure 64, nucleus

This trichonymphid is associated in its host with a species of *Stephanonympha* having an unusually large number of nuclei, *Oxymonas clevelandi* Zeliff, and *Tricercomitus divergens* Kirby.

It is closely related to *Trichonympha chattoni*, but differs from it at least in nuclear structure and in the endoplasmic inclusions. The chromatin is arranged in small vesicles, all of similar size, disposed in linear series or groups. Near the periphery is a small, globular nucleolus enclosed in a clear space. The granules, rods, and masses of chromatin in *T. chattoni* from *K. contracticornis* are solid, and do not show the vesicular structure even when destained to the point

where this is clearly visible in *T. tabogae*. The nucleolus in *T. tabogae* does not reach so large a size as in *T. chattoni*.

In the cytoplasm the conspicuous spherules and the large, haematoxylin-staining bodies in the endoplasm of *T. chattoni* are not present, though there are numerous smaller granules and spherules which correspond to the former of these. The spherules vary considerably in size in different individuals of *T. sphaerica,* and it may be that in some specimens of *T. tabogae* larger spherules would be encountered. But the difference in their size in series of specimens of *T. tabogae* and *T. chattoni,* both from wood-fed termites, is significant.

The parabasal apparatus was very obscure in the material available for study. From the traces of it which could be discerned, it seems to consist of cords arranged like those of *T. chattoni.*

Trichonympha quasilli sp. nov.

Host: *Kalotermes* (*Kalotermes*) *snyderi* Light. Costa Rica.

Plate 28, figure 42; plate 30, figures 57–58

The host termite of this species is the one identified by Banks (1918) as *Kalotermes marginipennis* Latreille and referred to under that name by Kirby (1928, 1931) and by Zeliff (1930). Light (MS) has pointed out that this was an incorrect determination, and has proposed the new name *K.* (*Kalotermes*) *snyderi* for the species, which ranges through Central America and Mexico into the southeastern part of the United States.

Besides an abundance of *Trichonympha quasilli, Kalotermes snyderi* contains a species of *Calonympha,* a simpler calonymphid of an undescribed genus, two or three genera of devescovinids, *Oxymonas panamae* Zeliff and *Tricercomitus divergens* Kirby.

The flagellate agrees closely in general appearance (pl. 28, fig. 42) with *Trichonympha chattoni,* the length ranging from 81 to 144μ, the width from 31 to 53μ, averaging 111 × 38μ. The rostrum is almost identical in size and form with that of *T. chattoni,* measuring in length in the center from 9.5 to 11μ, in diameter at the base of the cap from 6 to 7μ, and in diameter at the base of the collar from 12 to 14μ. The ratio of the flagellated to the non-flagellated region ranges from 0.29:1.00 to 0.62:1.00, averaging 0.44:1.00. The flagella are arranged as in most species of *Trichonympha,* gradually increasing in length posteriorly, those arising near the ends of the plates extending beyond the end of the body.

The middle zone of ectoplasm is thicker than in the case of *T. chattoni*, in which it is unusually narrow. In this respect *T. quasilli* agrees more closely with most other species of *Trichonympha* than with *T. chattoni*.

The distinctive characteristic of *Trichonympha quasilli* is the parabasal apparatus, which consists of numerous slender cords all of which converge just posterior to the nucleus (pl. 28, fig. 42). Anteriorly the parabasal cords pass close to the boundary of the endoplasm a short distance anterior to the nucleus, and forward from this point they cannot be traced. All, or almost all, the cords touch the membrane at the posterior part of the nucleus. Thus there is formed a basket-like structure, in the bottom of which the nucleus is situated; this led to selection of the specific name.

The nucleus (pl. 30, figs. 57–58), which measures from 8 to 13 microns in diameter, is situated near the posterior limit of the flagellated zone, so that it is usually somewhat less than a third of the distance from the anterior end. It contains many small granules 'of chromatin, isolated or arranged in groups, occupying almost all of the space within the membrane, and a spherical nucleolus often about two and one-half microns in diameter, in a clear space near the periphery. The nucleolus remains unstained after use of Delafield's haematoxylin, but it often stains deeply with iron-haematoxylin.

In the prenuclear endoplasm are numerous small granules, which are not so large or so conspicuous as those of *T. chattoni*. The postnuclear endoplasm contains numerous spherules similar to those of *T. sphaerica* and *T. chattoni*. Dr. Cleveland, who studied living material of *T. quasilli* from *Kalotermes snyderi* kept for six years in Boston, informed the writer that the spherules stained with neutral red.

Trichonympha subquasilli sp. nov.

Host: *Kalotermes clevelandi* Snyder. Panama.

Plate 28, figure 41; plate 30, figure 61

In *Kalotermes clevelandi*, associated with *Coronympha clevelandi* Kirby, *Oxymonas clevelandi* Zeliff, and *Tricercomitus divergens* Kirby, is a species of *Trichonympha* which agrees with that in *Kalotermes snyderi* in every respect except certain details in the arrangement of the parabasal apparatus and perhaps in the structure of the nucleus.

In both species, the parabasal cords are of the same size, and in *T. subquasilli* also they converge toward the posterior part of the nucleus, which most of them touch. They are, however, more sinuous, a greater number of them are free, and they do not form so regular a basket as do those of the preceding species. The arrangement of the parabasals in both species has been constant in the considerable number of specimens studied. It might seem that this is a matter of variation in material, but, except at times of division, the parabasal apparatus seems to possess a constant arrangement in each of the different species of *Trichonympha*.

The nucleolus is the same in both forms, but in the material studied there is a difference in the arrangement of the chromatin, which is more reticular in *T. subquasilli;* this, however, may be a result of fixation.

These small, but constant and unmistakable differences between the two forms, when considered in connection with the very considerable differences in the faunas as a whole, leave no choice other than the recognition of two species.

A *Trichonympha* corresponding in every way with *T. subquasilli* occurs in the undetermined species of *Kalotermes* from the Galapagos Islands from which *Coronympha clevelandi* was also recorded (Kirby, 1929). This termite has the same flagellate fauna as *Kalotermes clevelandi.*

Trichonympha lighti sp. nov.

Host: *Kalotermes (Kalotermes) emersoni* Light. Mexico.

Plate 27, figures 38–40; plate 30, figure 56

For material from this termite the writer is indebted to Dr. S. F. Light, who brought specimens from Colima, Mexico. Associated with *Trichonympha* are the following flagellates: *Stephanonympha* sp., a form resembling *Coronympha* but having eight instead of sixteen nuclei, *Oxymonas* sp., and *Tricercomitus divergens.*

In size and shape this species (pl. 27, fig. 38) closely resembles other species described from *Kalotermes*. The length in a number of specimens ranged from 94 to 138μ, the width from 36 to 65μ. The rostrum (pl. 27, fig. 39) measures in length in the center from 11 to 12μ, in diameter at the base of the cap from 6 to 8μ, and in diameter at the base of the collar from 11 to 13μ. The ratio between the flagellated and non-flagellated regions ranged in a number of cases from

0.36 :1.00 to 0.50 :1.00, averaging 0.42 :1.00, practically the same as that in *T. quasilli*. As in that species, the flagella increase gradually in length toward the posterior portion of the flagella-bearing plates, where there are very long ones which clothe the body and extend a short distance beyond the posterior end.

T. lighti also resembles *T. quasilli* in the thickness of the ectoplasm and the relative thickness of its layers; but the uniformity in their thickness from the anterior part posteriorly, the conical form of the anterior endoplasm and the abrupt outward extension of this at the base of the cone, is more suggestive of the condition in *T. sphaerica.*

The parabasal apparatus (pl. 27, figs. 38, 40) consists of about thirty to forty very slender, sinuous cords, none of which extend beyond the level of the posterior end of the nucleus. Some of them end freely beside or anterior to the nucleus, but many are so arranged that their posterior ends meet the nuclear membrane. Usually they do not extend around the nucleus, but meet it on the wall of its anterior half. There appears to be no firm attachment, however; in ruptured material the nucleus is sometimes entirely separated from the parabasal cords. After fixation in osmic vapor, the cords stain black with iron-haematoxylin.

The nucleus (pl. 30, fig. 56), which is situated just within the posterior limits of the flagella-bearing zone, is remarkably small, measuring only from six to eight microns in diameter. In all other described species of *Trichonympha* the nucleus is considerably larger, usually averaging at least twice the size of this. The chromatin material, which fills up the greater part of the space within the membrane, leaving only a narrow clear space, is dispersed in closely packed, varicose strands, which in optical section appear as granules.

The subrostral cone of endoplasm is densely packed with small, haematoxylin-staining granules (pl. 27, fig. 39), arranged like the similar group of granules in *T. quasilli.*

The postnuclear endoplasm contains, besides wood, numerous spherules resembling in size and abundance those in *T. chattoni, T. sphaerica,* and other species. There is also a large number of chondriosomes, shaped like those described from *T. chattoni* and the species from *Termopsis*. These stain with iron-haematoxylin after fixation in osmic vapor, but are not visible in material fixed in Schaudinn's fluid.

The peculiar arrangement of the parabasal apparatus and the unusually small size of the nucleus serve to distinguish this species from all other species described in this paper.

Trichonympha saepiculae sp. nov.

Hosts: *Kalotermes (Eugitermes) kirbyi* Snyder. Panama.
Kalotermes (Eugitermes) panamae Snyder. Panama.

Plate 25, figures 29–30; plate 26, figures 32–34; plate 30, figures 62–63; figure C

Associated with this species of *Trichonympha* in *K.* (*R.*) *kirbyi* is a species of *Calonympha,* a small calonymphid of an undescribed genus, *Oxymonas kirbyi* Zeliff and a small devescovinid, as well as *Tricercomitus divergens. K.* (*R.*) *panamae* has an apparently identical fauna. *Kalotermes panamae* was originally assigned to the subgenus *Kalotermes,* and has been referred to previously by the writer under that designation, but it has been ascertained by S. F. Light that the species really belongs in the subgenus *Rugitermes.* To this reassignment Snyder, who originally described the species, has agreed. According to Light there is no doubt that the two host species are different, despite the similarities in their faunas.

The length of the specimens from *K.* (*R.*) *kirbyi* (pl. 26, fig. 32) ranged from 82 to 149μ, those from *K.* (*R.*) *panamae* (pl. 25, fig. 29) from 72 to 144μ, averaging in all 112μ; the width of the former was from 29 to 62μ, from the latter from 29 to 66μ, averaging in all 48μ. The anterior flagellated region occupies about a third of the length of the body, the ratio ranging from 0.37:1.00 to 0.75:1.00, averaging 0.55:1.00. The rostrum (pl. 25, fig. 30), which is exactly like that of *T. quasilli,* measures in length in the center from 11 to 13μ, in diameter at the base of the cap from 6 to 8μ, and in diameter at the base of the collar from 11 to 13μ (17μ in one exceptional case).

The ectoplasm of the flagella-bearing region is not so thick and does not terminate as abruptly as in *T. sphaerica* and *T. lighti,* but the middle layer of ectoplasm is thicker than that of *T. chattoni.* Two-thirds or more of the ectoplasm consists of the outer layer, and the inner layer is thin and tapers off toward the posterior, unlike the situation in *T. sphaerica.*

The distinctive characteristic of this species of *Trichonympha* is the arrangement of the parabasal apparatus (pl. 26, figs. 32, 34). This consists of about forty slender cords, which may be traced to a position in the peripheral endoplasm of the anterior part of the body, and which form a cylinder (or hedge, whence the name *saepiculae*) of a single layer of parallel bars around the nucleus, to extend for some distance beyond it. The cords lie adjacent to one another throughout their length, as far anteriorly as they can be traced.

Between each two appears a narrow clear space, the breadth of which is even for the whole extent, and is uniform between all parabasals of the group. At the level where the cage-like structure surrounds the
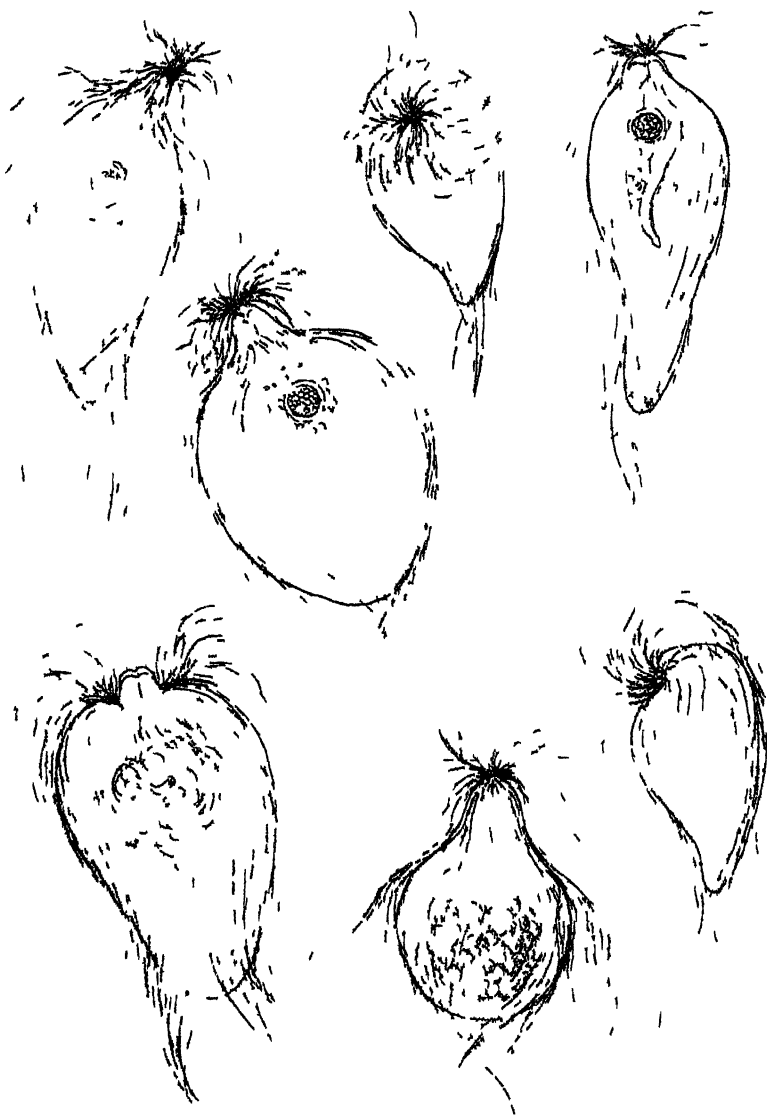
Fig. C.   *Trichonympha saepiculae* from *Kalotermes* (*Eugitermes*) *kirbyi.* Sketches from living animals, showing form and arrangement of flagella.   × approx. 425.

nucleus, the cords typically lie adjacent to the membrane at the region of greatest diameter, where the nucleus is sometimes elongated transversely, and where the cords are sometimes bent inward.

In the postnuclear portion the cords are generally more or less curved or bent, but there are groups in which the cords remain parallel to one another. The cylinder, however, is often split in places, and often the splits extend anteriorly. Sometimes, especially in the specimens from *K. panamae* (pl. 25, fig. 29), the parabasals are not arranged in a continuous cage, but in separate groups surrounding the nucleus in a similar manner.

The nucleus (pl. 30, figs. 62–63) is situated just within the limits of the flagellated zone, its posterior end being near the level of the ends of the plates and ridges. Its diameter ranges from ten to seventeen microns, averaging about thirteen microns. Within the membrane, filling the interior except for a narrow space beneath the membrane, is the chromatin arranged in irregular, varicose strands. These are much coiled, sometimes in a compact mass, sometimes more loosely disposed. The larger nuclei with dispersed strands are doubtless in premitotic stages. A small, peripheral nucleolus is often distinguishable.

In several instances intranuclear parasites have been observed (pl. 26, fig. 33). Spheroidal bodies filled the nucleus, and each, in some cases, appeared to be subdivided. This probably is not *Nucleophaga*.

The prenuclear endoplasm is filled with an abundance of small granules (pl. 26, fig. 34), the mass being enclosed by the anterior portions of the parabasals and extending posteriorly nearly to the nucleus. Posterior to the nucleus the endoplasm contains, besides wood, spherules similar in size, form, structure, and abundance to those which in *Trichonympha sphaerica* stain with neutral red.

In the postnuclear cytoplasm of *T. saepiculae* from *K. panamae* stained with iron-haematoxylin after Schaudinn's fluid are visible numerous small granules, single or arranged in linear series (pl. 25, fig. 29). At first sight, these suggest chondriosomes, but they differ from those usually present in *Trichonympha* in form and in their constant presence after the technique used. They are similar to the granules and rodlets shown by Kofoid and Swezy in their diagram of *T. campanula*, which possibly were endoplasmic inclusions of the same nature.

TRICHONYMPHA IN RETICULITERMES

Trichonympha agilis Leidy, 1877

Hosts: *Reticulitermes flavipes* Kollar. Eastern United States. Other species of
Reticulitermes.

Plate 29, figures 49–54; plate 30, figures 55, 65

One way in which the known faunas of termites of the genus
Reticulitermes differ from all other known protozoan faunas of ter-
mites is in their universal inclusion of the peculiar flagellates of the
family Pyrsonymphidae. This fact is mentioned to indicate the dis-
tinctive character of the faunas of this genus, as well as the similarity
among those of its species. There are about fourteen known species
of *Reticulitermes*, of which six or seven have been explored for Pro-
tozoa. In all of these the faunas are quite similar, and agree in the
presence, among other polymastigotes and hypermastigotes, of *Tricho-
nympha agilis.* Such excellent accounts of this species have been given
by Leidy (1877, 1881), Porter (1897), Grassi (1893, 1917), and Koid-
zumi (1921) that it is possible to add very little to its description.
The writer has studied it in *Reticulitermes flavipes, R. hesperus* and
R. tibialis, and an illustrated account is given in this paper for the
sake of comparison between the type species of the genus and other
less well-known forms described in the foregoing sections.

Leidy's accounts are concerned only with those features which can
be observed easily in unstained material. An excellent description of
the internal anatomy of the animal was published by Porter; this,
except for the omission of the parabasal apparatus, was considerably
more complete and accurate than Grassi's description, published a
few years previously, of which, apparently, Porter was unaware. In
fact, Porter's description is considerably better than most of the later
descriptions of *Trichonympha.* França (1916) gave a brief and in-
complete account of the same species, without being able to refer to
Porter's work, though he knew of it. Grassi (1917) gave a more
accurate and detailed account than that in the paper written in col-
laboration with Sandias (1893). Koidzumi (1916, 1921) overlooked
some of the details which had previously been accurately described by
Porter and by Grassi. The division process in *Trichonympha agilis*
was described by Foa (1904).

In a number of papers published in 1919–1921 De Mello described
T. agilis from *Leucotermes indicola,* two species of *Coptotermes* and

Hodotermes viarum. It is clear that in the first three cases he mistook *Pseudotrichonympha* for *Trichonympha.* *Hodotermes viarum* possibly contains *Trichonympha,* but almost certainly not the species *agilis.* He also (1920*b*, 1921*b*) claimed to have identified some of Bugnion's figures of flagellates from *Coptotermes travians, C. flavus* and *Termitogeton umbilicatus* as representing *Trichonympha agilis,* though doubtless here also *Pseudotrichonympha* was concerned.

De Mello (1921*a*) published a paper on the trichonymphids of *Leucotermes indicola,* which he had previously reported upon before the Indian Science Congress meeting January 13–18, 1919, and in a brief published note (1919). In this paper he reproduced the figures by Leidy, but the reproductions lack the comparative accuracy of the originals. He correctly pointed out, as others had done before, that the so-called young forms which Leidy ascribed to *T. agilis* do not belong to the same genus. His detailed criticism of Leidy's figures and descriptions are, however, of little value, since they were made on the basis of observations, in themselves incorrect, on *Pseudotrichonympha,* which he then supposed to be *Trichonympha agilis.* De Mello later (1927, 1928) revised his work on the flagellates of *Leucotermes indicola.*

Trichonympha agilis is usually less abundant in its hosts than are the species of *Trichonympha* in *Termopsis.* It is smaller than any other species which has been observed, except that in *Hodotermopsis;* in *Reticulitermes* it is smaller than many individuals of *Pyrsonympha.* Leidy found the length to range from 75 to 115μ, the breadth from 30 to 45μ. In living material from *R. tibialis* fed for some time on filter paper, the writer has recorded lengths from 55 to 84μ, widths from 22 to 40μ, averaging 74 × 29.5μ. Fixed material from *R. flavipes* ranged from 54 to 110μ in length, and from 23 to 55μ in width, averaging 78 × 36μ. In the living material the ratio of the flagellated to the non-flagellated regions ranged from 0.44:1.00 to 0.81:1.00, averaging 0.64:1.00, or about two-fifths of the total length. In fixed material from *R. flavipes,* however, the ratio often appears much less than this.

Although in the normal condition (pl. 29, fig. 49), in living specimens, the length exceeds twice the greatest width, as in almost all other species of *Trichonympha,* in fixed material the body is often relatively much broader and the posterior end more rounded. Koidzumi has distinguished the variety *japonica* in *Reticulitermes speratus,* from which he did not record the typical form of *T. agilis,* as being

unusually broad, from 40 to 70μ in animals of from 70 to 90μ in length, and having a flagellated zone "occupying some one-fourth of the total length." This unusual breadth, however, is by no means characteristic of *Trichonympha,* so it seems likely that Koidzumi was misled by the study of abnormal fixed material. Since in the only figure of this supposed variety which he has published the flagellated zone occupies slightly more than a third of the total length, it appears that this characteristic falls within the range of *T. agilis.*

The variety *formosana* was described by him from the termite *R. flaviceps* of Formosa, which is generally believed to be a synonym of *R. speratus.* In this the shape is that of *T. agilis,* but the flagellated zone is said to occupy only about one-sixth of the body length. His figure of this variety appears to represent an animal in which the labile posterior cytoplasm is abnormally drawn out, as is often the case in fixed material. As these two varieties are not given adequate definition, it seems to the writer advisable to reject them.

The shape and movements of the flagellate have been admirably described and illustrated by Leidy (1881). An accurate idea of its form can be obtained only from fresh, active material, for this is usually considerably altered in fixed or even in moribund specimens. The body in general is more or less spindle-shaped, with the widest portion at or anterior to the middle (pl. 29, fig. 49). At the anterior end is the evenly rounded cap, while the variable posterior end may be tapered and truncated, rounded or pointed, or may be broadly rounded with little taper of its sides. Often the body bulges out slightly posterior to the end of the flagellated zone, a fact which led Leidy to consider it to be divided into two parts like *Gregarina.*

The rostrum (pl. 29, fig. 54), called the nipple by Porter, Grassi, and Koidzumi, is identical in structure with that of other species of *Trichonympha,* but is wider at the base than in the larger species from *Kalotermes.* Measurements of the rostrum in living material gave the following ranges: length in center from 9.5 to 12μ; diameter at base of cap from 7 to 8.5μ, diameter at base of collar from 18 to 21μ. In some fixed material, however, rostra of smaller size were encountered; the one from which figure 54, plate 29, was drawn had a length in the center of 9.6μ, a diameter at the base of the cap of 6μ, and a diameter at the base of the collar of 14μ.

The rostrum of *T. agilis* has not been represented with perfect accuracy in any published accounts. The cap has been correctly shown in most of them; this is collapsed in many fixed specimens, and

when partly deformed has the form of a brimmed hat shown by Grassi in some of his figures (1917, pl. 4, figs. 2*b*, 3). The blepharoplast, which appears in unstained material as a highly refractive granule that is not quite a hemisphere, is convex anteriorly and flat posteriorly, where it is met by the constricted end of the rostral tube. Porter and Koidzumi observed it incorrectly as merely a knob-like enlargement of the rostral tube, which they respectively called the axial rod and axial core. França(1918) represented it more accurately than did the other authors. He (1916) was the first to apply the term blepharoplast, though he designated by this name the ensemble of the granule and the rostral tube, which latter he observed, doubtless in overstained material, as solid. Later (1918) he designated the so-called mushroom-shaped structure (blepharoplast and rostral tube) as the blepharoplast plus the parabasal body, apparently incorrectly identifying the rostral tube with the parabasal of *Devescovina.*

The rostral tube is narrow, much narrower than shown by Koidzumi in his (1921) plate 10, figure 13, and is constricted in its anterior portion before it meets the blepharoplast. Surrounding it is the dense inner layer of ectoplasm which coincides anteriorly with the edges of the blepharoplast. This was shown by Porter, who correctly recognized the three layers of ectoplasm in the rostrum, though Koidzumi credited him with defining only the inner and outer layers. The outer layer is at least twice as thick as the other two layers combined, which consitute the inner layer as defined by Koidzumi.

Posterior to the rostrum is the fissure which was recognized by other authors. Grassi erroneously described it as a circular cavity filled with a liquid and separated from the surface by a pellicle, calling it the cytarthrosis; actually it is an open fissure and the contents of the inner space must be the liquid in which the flagellate moves. Porter observed the collar lifted from the anterior portion of the body (1897, pl. 1, fig. 4), as described and illustrated in *T. collaris* in this paper (pl. 21, fig. 3).

The anterior, flagella-bearing portion of the body (pl. 29, fig. 50) was designated as the middle or bell-shaped part by Porter, the bell by Koidzumi, and the posterior part of the middle or flagella-bearing zone by Grassi. The ectoplasm is relatively thick and ends abruptly posteriorly, as in *T. sphaerica,* without decrease in thickness. The inner layer is very thin and scarcely visible. Porter incorrectly considered the middle layer of the body to be the equivalent of the inner

layer of the rostrum. The fact that the transparent middle layer is traversed by the roots of the flagella can be clearly seen both in living and fixed material. Koidzumi, nevertheless, did not observe this fact, though he mentioned the description by Porter of the flagella traversing the transparent layer, but erroneously described the origin of the flagella to be from the region between the outer and the transparent layer.

In the anterior flagella-bearing portion of the body, the outer layer is about twice the thickness of the other two layers combined. This layer contains the plates which were described by Porter, who used the terms ridges and plates as synonymous. In his cross-sections, eighty-two plates are shown in the body region and forty-two in the rostrum. In one specimen of *T. agilis* from *R. tibialis*, the writer counted thirty plates in the rostrum. Grassi called these plates myonemes or little ribs, and the term myonemes was adopted by Duboscq and Grassé (1927b). There is, however, no evidence that they are contractile. No peripheral granules like those in *T. collaris* are present between the plates.

Just posterior to the rostral fissure is a dense disc or series of bands extending outward from the layer of basal granules, and closing anteriorly the fluid middle layer (pl. 29, fig. 54). Grassi showed traces of this (1917, pl. 4, figs. 4a, 4d, 7, 25a), but it was overlooked by Porter and Koidzumi. França described this in 1916, and showed it in a drawing in 1918 as a siderophile plate at the anterior border of what he called the third segment of the body. This he believed to be continuous with the mushroom-shaped structure, that is, with the rostral tube. Since he apparently observed it in overstained material, he did not describe the details of its structure.

Jírovec (1931) presents a microphotograph of a specimen of *Trichonympha* from *Reticulitermes lucifugus* which he had impregnated with silver by a modification of Klein's method. This shows what he states to be parallel rows of basal granules, but which are doubtless really the plates. The plates can be demonstrated quite as well, or better, by ordinary methods of preparation.

The basal granules are minute granules situated close to the boundary of the endoplasm. Grassi believed that there are also granules at the peripheral ends of the basal fibers, between the middle and outer layers; and Koidzumi states that this region is deeply staining, though he does not describe granules in it. It is probable that there are no definite granules there, but that Grassi's statement is based

on the existence of enlargements of the flagellar roots at or near the inner ends of the plates (cf. *T. magna,* p. 392).

The arrangement of flagella (pl. 29, fig. 49) is well indicated in Leidy's figures, though he was incorrect in believing that these arise in three or four circles. On the rostrum the numerous flagella are all of about an equal length of from 25 to 30μ, except that just posterior to the cap there are sometimes a few short ones of about five microns. These very short ones appear not to be always present, according to observations by the writer, but they have been seen in living specimens and are figured by Leidy and Grassi. Posterior to the rostrum the flagella increase in length to the long posterior ones which often extend from 20 to 30μ beyond the posterior end. These longest ones, which run over the surface of the body, do not pursue an exactly longitudinal course, but are directed slightly laeotropically. Beyond the end of the body these often form what Leidy described as ''a twisted fasciculus with divergent ends,'' which he represented faithfully in his drawings. The twist is always laeotropic, the flagella slanting on the upper surface from the left anterior to the right posterior. The writer has made observations confirming the statement of Porter that *T. agilis* often attaches itself by the ends of the group of long flagella. Thus it may be held fast to some object, or may swim about carrying material attached to the flagella. Within this fascicle, wood particles and other material often may be entangled and, as described by Porter, be conveyed within a cavity formed in the posterior end of the body.

The parabasal apparatus of *Trichonympha agilis* (pl. 29, figs. 51–53) has been known since Grassi and Sandias (1893) described the structure as a sort of little basket (''cestello'') composed of curved rods ''rather remote from one another and cemented together by a granular protoplasm,'' extending inward from the posterior part of the flagellated zone to embrace the lower part of the nucleus. Porter, who apparently did not know of this observation of Grassi, failed to describe the rods, but did observe the position of the ''cestello'' as a granular, bowl-shaped partition. Koidzumi (1921), who proposed the name ''corbule'' for the structure, believed it to be a continuous membrane the inner surface of which is lined by granules. Doubtless he observed the parabasal cords, but misinterpreted them as optical sections of a membrane.

As noted by Grassi (1917), the ''rodlets'' are considerably less numerous than the plates. They are slender cords, relatively much

smaller than those in *T. saepiculae*. They first appear in the periphery of the endoplasm at the posterior end of the flagellated zone. Typically the parabasal cords extend inward, forming a bowl-shaped structure (pl. 29, fig. 51) within which is the nucleus, as in *T. quasilli* (pl. 28, fig. 42). The ends of the cords usually are situated against the posterior part of the nuclear membrane, and apparently they do not meet one another to form a complete basket. There are, as noted also by Koidzumi, variations from this typical arrangement. In some cases (pl. 29, fig. 52) the parabasals are closely approximated to the sides of the nuclear membrane, and sometimes the nucleus has slipped back so as to be almost free of the basket. In other cases (pl. 29, fig. 53) the nucleus is more anteriorly situated, and the ends of the parabasals extend freely behind it. These variations in arrangement, as well as the relations observed between the cords and the nuclear membrane, make it seem improbable that the cords are actually firmly attached to the membrane. It is improbable, also, that the cords are attached to one another, either ''cemented together by a granular protoplasm'' (Grassi and Sandias, 1893) or connected by a membrane (Koidzumi, 1921).

The nucleus (pl. 30, fig. 65), which ranges from about 10 to 14μ in diameter, is situated near or just beyond the posterior end of the flagellated zone. As stated by Grassi (1917), it is in shape spheroidal or ellipsoidal with the long axis in a transverse direction. In it there is a small, peripheral nucleolus and a mass of chromatin filling all but a limited space under the membrane. The chromatin appears to be arranged in separate granules and compact masses more generally than is the case in the nuclei of most species of *Trichonympha*.

The anterior endoplasm contains a great number of closely packed granules (pl. 29, fig. 50; pl. 30, fig. 55), which are relatively larger than those in the same situation in *T. campanula*. These fill the entire space delimited posteriorly by the parabasal apparatus, the position of which can be clearly seen in living material as a clear border (pl. 29, fig. 50) separating the anterior from the posterior endoplasm. The anterior endoplasm is well defined in living material as a dense, granular, ellipsoidal mass from the anterior end of which arises the rod-like core of endoplasm in the rostral tube. Grassi and Sandias (1893, pl. 5, fig. 1) represented this mass, though in their figure its form is incorrect. This incorrect drawing is, unfortunately, the one usually reproduced in compilations to illustrate *T. agilis*. Porter (1897, pl. 1) correctly represented the form and appearance of the mass. The

granules in the anterior endoplasm are preserved in material fixed in Schaudinn's fluid with acetic, Flemming without acetic, osmic vapor, and other fluids, and may be stained with iron-haematoxylin after any of these, but especially deeply after osmic vapor (pl. 30, fig. 55).

In the posterior endoplasm are numerous granules similar in size and chemical behavior to those referred to in the preceding paragraph, but they are more scattered and there are also very many smaller ones. Among them are food bodies, especially wood particles, in vacuoles. In some material from *R. tibialis* fixed in osmic vapor and stained in iron-haematoxylin, there appeared a broad, very densely granular region across the body at the level of the nucleus (pl. 30, fig. 55).

Neutral red staining shows no conspicuous spherules. Some particles of wood in the body may take the stain, and among the many minute endoplasmic granules posterior to the nucleus are a few which stain red. The condition is in marked contrast to that in the associated flagellates of the genera *Dinenympha* and *Pyrsonympha,* which contain many neutral red staining spherules.

Sphaerita frequently has been found parasitizing *Trichonympha agilis.* Leidy figured (his pl. 51, fig. 7) an animal containing "two large, coarsely granular balls, supposed to be masses of spores," which were doubtless sporangia of *Sphaerita.* Porter (his pl. 2, fig. 10) showed one of the parasites, which he regarded as spores of fungi ingested as food. The writer has seen numerous instances of parasitism, including an especially heavy infection shown to him by Dr. Virginius Brown in which *Sphaerita* was present in a large percentage of the flagellates in a colony of termites, several sporangia sometimes occurring in one *Trichonympha.*

In some specimens of *T. agilis* from *R. tibialis* fixed in osmic vapor, rod-like bodies were present in the cytoplasm of all individuals on several slides (pl. 30, fig. 55). These varied in length, and some appeared to be dividing transversely in the middle. Probably these represented an organism parasitic in the flagellates of that colony of termites, as they have not been seen in other material.

Georgevitch (1930) described as a new species *Trichonympha serbica* from *Reticulitermes lucifugus* of Jugoslavia. He asserts that the principal constituents of the fauna of this termite differ notably from the flagellates of the termites of the same species from Italy and France, and he also described new species of *Pyrsonympha* and *Spiro-*

trichonympha. It is improbable that the faunas of *R. lucifugus* differ in the two localities, for many observations have shown no differences in the faunas of given species of termites according to distribution. His brief, unillustrated description of *T. serbica* agrees in every point with the description of *T. agilis* above. He makes no mention of *T. agilis*, but only of *T. minor*, and states that his species differs from it in form, parabasal apparatus, and habitat. He wrongly states the host of *T. minor* to be *Coptotermes sjöstedti*, probably having been misled by Grassi's table which lists *Pseudotrichonympha hertwigi* var. *minor* from that termite, but which fails to list *Trichonympha minor* of *R. lucifugus*. *Trichonympha serbica* is probably a synonym of *T. agilis*.

Trichonympha minor Grassi and Foa, 1911

Host: *Reticulitermes lucifugus* Rossi. Europe.

This species, which is reported to be associated with *T. agilis* in *R. lucifugus*, has not been seen by the writer. It was named and briefly characterized by Grassi and Foa (1911) and more fully described and illustrated by Grassi (1917). As these accounts give no very specific characteristics, it is difficult to diagnose the species. Grassi states that it is a little smaller than *T. agilis*, has a much shorter flagella-bearing zone, a smaller nucleus which is closer to the anterior end, more closely approximated and presumably more numerous ribs (plates), a more finely granular anterior endoplasm, and a differently arranged basket (parabasal apparatus). The parabasal cords do not, according to Grassi's figures, all touch the nuclear membrane, and in all cases they extend for some distance beyond the nucleus, slanting laeotropically.

In the position of the nucleus and the extent of the flagellated zone, *T. minor* appears to be related to *T. agilis* somewhat as *T. sphaerica* is to *T. campanula*. The studies reported in this paper indicate that the arrangement of the parabasal cords is a good diagnostic characteristic in *Trichonympha*. It seems that in this respect also there is a valid point of distinction between *T. minor* and *T. agilis*. It is, however, very desirable that the species be studied more completely and its characters more adequately defined so that it can be better compared with *T. agilis* and the other species of the genus.

TRICHONYMPHA-LIKE HYPERMASTIGOTES OF
UNCERTAIN POSITION

Under this heading will be considered three species of hyper-mastigotes which have been assigned to the genus *Trichonympha* by the original authors or other writers, but which lack adequate descriptions. These are *Trichonympha leidyi* Kent, *Leidyonella cordubensis* Frenzel, and *Gymnonympha zeylanica* Dobell.

Trichonympha leidyi Kent, 1885

Host: Unnamed termite from Tasmania.

There is no possibility of determining the specific status of this flagellate, of which Kent gives only a brief, unillustrated account. Kent described the organism as elongate or pyriform, longitudinally striated, with short flagella clothing the entire surface of the body. He observed what he believed to be an oral aperture in the form of a transverse slit situated at a short distance from the anterior end, on one side of the body, but certainly this could not have been an oral aperture.

Associated with the flagellate Kent found what he believed to be young forms, some "of an ovate contour, and clothed throughout with cilia of an even length," others elongate with "the surface . . . . obliquely furrowed in opposite directions." The former may have been *Holomastigotoides;* the latter were possibly *Spirotrichonympha*. Kent claimed to have observed transverse fission of the body in these forms; this, however, may have been merely the sloughing off of part of the cytoplasm which sometimes occurs in moribund material.

The elongate form and covering of short flagella of the supposed adult forms of *Trichonympha leidyi* suggest its possible identity with *Pseudotrichonympha* (fig. D), as pointed out by Duboscq and Grassé. It seems likely, in so far as one can form an opinion from Kent's meager account of the fauna, that the termites he examined belonged either to the genus *Coptotermes* or *Leucotermes*. The flagellates in termites of these genera belong to the genera *Pseudotrichonympha, Holomastigotoides,* and *Spirotrichonympha*. Kent reports that he observed two other flagellates, one of which he states was apparently

Pyrsonympha, while the other was *Lophomonas.* *Pyrsonympha* has been found only in *Reticulitermes,* which has not been found in Tasmania, so this record is probably incorrect; and his determination of *Lophomonas* is questionable.
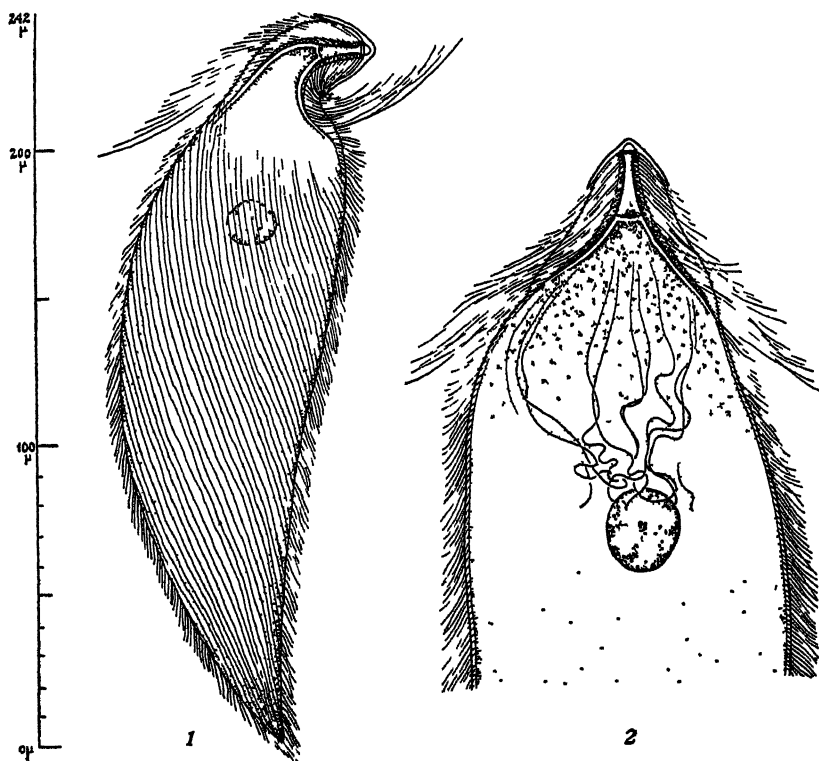
Fig. D. *Pseudotrichonympha* sp. from *Leucotermes aureus* Snyder of southern California. 1. Diagram to show the lacotropic striations from which the short body flagella arise over the entire posterior portion, and the general form. × 405. 2. Diagram of anterior end. The thick anterior ectoplasmic zone differs from that of *Trichonympha* in lacking the clear middle layer and having no rostral fissure. The structure corresponding to the rostral tube is closed posteriorly, so that the body endoplasm does not continue into it. There are parabasal cords and anterior endoplasmic granules similar to those in *Trichonympha.* × 530. Champy-Kull.

Leidyonella cordubensis Frenzel, 1891

Host: termite from Cordoba, Argentina.

Frenzel's paper states that this termite resembled *Eutermes inquilinus,* the name given by Fritz Müller to a Brazilian termite which, unfortunately, he did not describe sufficiently for subsequent identification. It is very improbable, however, that Frenzel's termite

belonged to the Termitidae, as does *"Eutermes"*; the presence of an abundant flagellate fauna of the type described indicates that it was probably one of the Kalotermitidae. From his description, *Leidyonella cordubensis* is not identifiable, unless it be upon further study by a process of elimination among the flagellate faunas of termites found in the vicinity of Cordoba, Argentina.

Duboscq and Grassé (1927*b*) have reviewed the suggestions of students of termite Protozoa concerning the genus to which Frenzel's flagellate probably belongs, and have concluded by placing it in the genus *Trichonympha*. Doubtless it corresponds more closely to this than to any other described genus from termites, but the possibility remains that it is generically distinct from *Trichonympha*. This, however, cannot be determined without further study of the organism.

In external form, longitudinal striation of the anterior portion of the body, length and arrangement of flagella, and position of the nucleus this flagellate has characters agreeing with those of *Trichonympha*. Frenzel gives the length as from 200 to 450μ, according to which the organism is larger than any known species of *Trichonympha* from termites. Frenzel's account gives two points of distinction from *Trichonympha*. He did not observe a thick anterior ectoplasm which is characteristic of the genus *Trichonympha*, though he noted Leidy's observation of a thick ectoplasm in the anterior portion of *T. agilis*. He did, however, describe a thick cuticle, and in this observed, in disorderly arrangement, numerous bacilliform rods which extended over the whole peripheral region of the body except the anterior papilla.

Gymnonympha zeylanica Dobell, 1910

Host: *Kalotermes (Neotermes) militaris* Desneux. Ceylon.

This flagellate was given a brief and inaccurate description by Dobell, but fortunately its host is known. It is clear that, as Duboscq and Grassé have pointed out, it is a *Trichonympha*. Nevertheless, the genus *Gymnonympha* has been given recognition in textbooks (Doflein-Reichenow, 1928; Kudo, 1931) and in the former Dobell's inaccurate figure has been reproduced. The writer has been able to examine, in alcoholic specimens of *K. militaris* from Ceylon, flagellates which probably belong to the species described by Dobell.

The generic distinction of the genus *Gymnonympha* is based on the facts that the flagella are few, arise all from a single ring around the base of the vesicular cap, and are only about half the length of the

body. The maximum length of the animal is about 150μ. The small conical process described by Dobell at the anterior end is doubtless the result of poor preparation of the structures here situated in *Trichonympha*. The series of striations, extending about one-third of the length of the animal, are apparently the plates, from which in all other species flagella are known to arise.

The *Trichonympha* observed by the writer in *Kalotermes militaris* corresponded in size and general structure to those described from other species of *Kalotermes* in this paper, and with *G. zeylanica* except for the arrangement of flagella, which were disposed as in other species of the genus. In stained material it is quite possible to overlook the flagella of the more posterior portion, while the shorter rostral flagella are clearly visible. Probably Dobell was misled into this error.

Dobell's description of *G. zeylanica* is not complete enough for comparison of it with other species of *Trichonympha*.

GENERAL ACCOUNT OF THE GENUS TRICHONYMPHA

Distribution of *Trichonympha* in Termites

In order to illustrate the character of the distribution of *Trichonympha* records are given below of the occurrence of species of the genus in 111 species of termites. In all cases, of course, other flagellates are present, but only *Trichonympha* is reported in this list. The records have been derived from three sources: the reports of other authors, the examination of smears in the possession of the writer, and a survey of the infections of termites preserved in alcohol. For the alcoholic material examined, the writer is indebted to Dr. S. F. Light and Mr. G. F. Hill, and for critical aid in preparation of the list of termites, to Dr. S. F. Light.

In the following table are reported the names of the termites, the localities from which the termites examined were collected, and the presence or absence of *Trichonympha*, together with the names of the species which have been described. Notes on some of the infections follow this report, and in the next section of the paper is a comparative account of the species in the different groups of the insects.

Family MASTOTERMITIDAE Froggatt

Mastotermes Froggatt
  darwiniensis Froggatt   Australia   absent

Family KALOTERMITIDAE Banks

Subfamily HODOTERMITINAE Holmgren

Hodotermes (Hodotermes Hagen)
  mossambicus (Hagen)   South Africa   absent
Hodotermes (Anacanthotermes Jacobson)
  murgabicus Vasiljev   Turkestan   *Trichonympha
                                                      turkestanica*
  macrocephalus Desneux   India   *T. turkestanica*

Subfamily TERMOPSINAE Holmgren

Archotermopsis Desneux
  wroughtoni Desneux   India   absent
Termopsis Heer
  angusticollis Hagen   California, U. S. A.   *T. campanula
                                                      T. collaris, T. sphaerica*
  nevadensis Hagen   California, U. S. A.   *T. campanula
                                                      T. collaris, T. sphaerica*
  laticeps Banks   Arizona, U. S. A.   *T. campanula*
Hodotermopsis Holmgren
  japonicus Holmgren   Amami-Ōshima, Japan   *Trichonympha* sp.

Subfamily STOLOTERMITINAE Holmgren

Stolotermes Hagen
  victoriensis Hill   Australia   absent

Subfamily KALOTERMITINAE Holmgren

Porotermes (Hagen)
  adamsoni (Froggatt)   Australia   *Trichonympha magna*
  grandis Holmgren   Australia   *T. magna*
Kalotermes (Kalotermes Hagen)
  aethiopicus (Silv.)   Eritrea, Africa   not reported
    =Epicalotermes aethiopicus Silv.   (Grassi, 1917)
  clevelandi Snyder   Panama   *T. subquasilli*
  contracticornis Snyder   Costa Rica   *T. chattoni*
  emersoni Light   Colima, Mexico   *T. lighti*
  flavicollis Fabricius   Europe   absent
  hubbardi Banks   Arizona, U. S. A.   absent
  immigrans Snyder   Hawaii; Marquesas;   *Trichonympha* sp.
                               Jarvis Island
  immigrans Snyder   Fanning Island   absent
  jouteli Banks   Mazatlan, Mexico   absent
  lighti Snyder   Arizona, U. S. A.   absent
  marginipennis (Latreille) Light Guadelajara, Mexico absent
    =K. montanus Snyder + K tuberculifrons
      Snyder; not K. marginipennis Banks, 1918
  mcgregori Light   Philippines   absent
  minor Hagen   California, U. S. A.   absent
  obscurus (Walker) Hill   Australia   *Trichonympha* sp.

occidentis Walker	Lower California, Mexico	*Trichonympha* sp.
platycephalus Light	Colima, Mexico	absent
rufinotum Hill	Australia	*Trichonympha* sp.
snyderi Light	Panama	*T. quasilli*
= *K. marginipennis* Banks, 1918		
tabogae Snyder	Panama	*T. tabogae*
taylori Light	Philippines	*Trichonympha* sp.
Kalotermes (*Neotermes* Holmgren)		
castaneus Burmeister	Florida, U. S. A.	absent
connexus Snyder	Hawaii; Marquesas; Tahiti	absent
erythraeus Silvestri	Eritrea, Africa	absent
gestri Silvestri	St. Thomas Island	not reported (França, 1918)
grandis Light	Philippines	absent
greeni Holmgren	Ceylon	absent
holmgreni Banks	Panama	absent
insularis White	Australia	absent
koshunensis Oshima	Formosa	absent
laticollis Holmgren	Seychelles	absent
longipes Froggatt	Australia	absent
malatensis Oshima	Philippines	absent
militaris Desneux	Ceylon	*T. zeylanica*
papua Desneux	New Britain Archipelago	absent
parviscutatus Light	Philippines	absent
rainbowi Hill	Funafuti	absent
samoanus Holmgren	Samoa	absent
schultzei Holmgren	Samoa	absent
zuluensis Holmgren	Zululand, Africa	absent
Kalotermes (*Metaneotermes* Light)		
russelli Light	Marquesas	absent
Kalotermes (*Paraneotermes* Light)		
simplicicornis (Banks)	Arizona, U. S. A.	absent
Kalotermes (*Rugitermes* Holmgren)		
kirbyi Snyder	Costa Rica	*T. saepiculae*
panamae (Snyder)	Panama	*T. saepiculae*
Kalotermes (*Glyptotermes* Froggatt)		
barbouri Snyder	Panama	absent
borneensis Haviland	Borneo	*Trichonympha* sp.
chapmani Light	Philippines	absent
fuscus Oshima	Bonin Islands	absent
iridipennis Froggatt	Australia	*T. chattoni* .
nigrolabrum Hill	Australia	*Trichonympha* sp.
parvulus Sjöstedt	Gold Coast, Africa	Not reported (Grassi, 1917)
satsumensis (Matsumura)	Japan	*Trichonympha* sp.
trilineatus Mjöberg	Australia	*Trichonympha* sp.
xantholabrum Hill	Tahiti	absent
Kalotermes (*Cryptotermes* Banks)		
breviarticulatus Snyder	Panama	absent
brevis Walker	Porto Rico; Colima, Mexico	absent
campbelli Light	China	absent

cynocephalus Light	Philippines	absent
dolei Light	Marquesas	*Trichonympha* sp.
dudleyi Banks	Panama	absent
grassii Silvestri. MS.	Chile	absent
havilandi Sjöstedt	Africa	not reported (Grassi, 1917)
hermsi Kirby	Fanning Island	absent
kotoensis Oshima	Formosa	absent
Kalotermes (*Planocryptotermes* Light)		
nocens Light	Philippines	absent
Kalotermes (*Calcaritermes* Snyder)		
brevicollis (Banks)	Panama	absent
emarginicollis Banks	Costa Rica	absent
parvinotus Light	Colima, Mexico	absent
Kalotermes (*Lobitermes* Holmgren)		
longicollis Banks	Panama	absent

Family RHINOTERMITIDAE Light

Subfamily PSAMMOTERMITINAE Holmgren

Psammotermes Desneux		not examined

Subfamily LEUCOTERMITINAE Holmgren

Leucotermes Silvestri		
aureus Snyder	California, U. S. A.	absent
clarki Hill	Australia	absent
indicola Wasmann	India	absent
occiduus Hill	Australia	absent
paradoxus (Froggatt)	Australia	absent
philippinensis Light	Philippines	absent
tenuis Hagen	Panama	absent
validus Hill	Australia	absent
Reticulitermes Holmgren		
flavipes Kollar	Eastern U. S. A.	*T. agilis*
fukienensis Light	China	*T. agilis*
hesperus Banks	California, U. S. A.	*T. agilis*
lucifugus Rossi	Europe	*T. agilis, T. minor*
speratus Kolbe	Japan	*T. agilis*
flaviceps Oshima	Formosa	*T. agilis*
tibialis Banks	California, U. S. A.	*T. agilis*

Subfamily COPTOTERMITINAE Holmgren

Coptotermes Wasmann		
acinaciformis (Froggatt)	Australia	absent
formosanus Shiraki	Formosa	absent
hartmanni Holmgren MS.	Brazil	absent
lacteus Froggatt	Australia	absent
michaelsoni Silvestri	Australia	absent
niger Snyder	Panama	absent
raffrayi Wasmann	Australia	absent
sjöstedti Holmgren	French Guinea	absent
Coptotermes sp.	Daman, India	⎫ *T. agilis*
Coptotermes sp.	Pragana, India	⎬ erroneously reported (Mello, 1920c)

Prorhinotermes Silvestri
   molinoi Snyder   Panama   absent

Subfamily TERMITOGETONINAE Holmgren

Termitogeton Desneux
   planus Haviland   Borneo   absent

Subfamily RHINOTERMITINAE Froggatt

Parrhinotermes Holmgren
   aequalis (Haviland)   Borneo   absent
   queenslandicus Mjöberg   Australia   absent
   inaequalis (Haviland)   Borneo   absent
Rhinotermes Hagen
   nasutus Perty   British Guiana   not reported
                                    (Dunkerly, 1923)
   reticulatus (Froggatt)   Australia   absent
Schedorhinotermes Silvestri
   intermedius Brauer   Australia   not reported
                                    (Grassi, 1917)
   putorius Sjöstedt   French Guinea   not reported (Grassi,
                                    1917; Dogiel, 1922)

Subfamily SERRITERMITINAE Holmgren

Serritermes Wasmann   not examined

Below are given some general notes on the distribution of *Tricho-nympha* in termites, with comments on some of the other Protozoa, leaving to the following section a correlative account of the speciation of the genus and the classification of termites.

In *Mastotermes*, *Trichonympha* is not present. What appears to be the only large, if not the only protozoan is a large hypermastigote of an undescribed genus, which has some resemblance to *Tricho-nympha* in the distribution of flagella, but has a uniformly thin ectoplasm and other important differences.

Of the seventy-eight species of Kalotermitidae listed above, twenty-seven contain *Trichonympha*. There is, however, not a great deal of uniformity in the distribution of the genus in this family of termites. In many of the more primitive members, namely, the subgenus *Anacan-thotermes* of the genus *Hodotermes*, *Termopsis*, *Hodotermopsis*, and *Porotermes*, *Trichonympha* has been found in all faunated specimens examined, but it was absent from those specimens investigated in the subgenus *Hodotermes* s. str., *Archotermopsis*, and *Stolotermes*. *Stolo-termes victoriensis* appears to contain only two Protozoa, namely, *Trichomonas* and a moderate sized hypermastigote of an undescribed genus, which has a distant relationship to the *Spirotrichonympha* group.

In *Hodotermopsis japonicus* the species of *Trichonympha* is relatively small in size, being comparable in that respect to T. *agilis*. One specimen was 96μ long, and while many were smaller than this, few exceeded it. In the extent of the flagellated zone, the position and size of the nucleus, and the position of the parabasal cords, in so far as this could be observed in alcoholic material, it is of the *T. agilis* type. While fairly numerous in the one nymph examined, it was by no means as abundant as *Trichonympha* in *Termopsis*. No other Protozoa could be recognized, but there may have been one or two small ones.

Among the subgenera of *Kalotermes*, *Trichonympha* is represented in ten of nineteen species of *Kalotermes* s. str., in both of two species of *Rugitermes,* and in five of ten species of *Glyptotermes*. On the other hand, only one of nineteen species of *Neotermes*, one of ten species of *Cryptotermes*, and none of one species each of *Metaneotermes, Paraneotermes, Planocryptotermes, Lobitermes,* and three of *Calcaritermes* contained a representative of the group. From the results of these examinations it appears likely that one may scarcely expect to find *Trichonympha* in *Neotermes* or closely related genera, or in *Cryptotermes* or closely related genera, while in *Kalotermes* s. str., *Rugitermes,* and *Glyptotermes* there is more probability of its being found present.

Among the Rhinotermitidae, *Trichonympha* has been found only in *Reticulitermes*. De Mello (1920c) reported it from two species of *Coptotermes* in India, but it is evident from the context and his later work that he mistook *Pseudotrichonympha*. In *Reticulitermes* it has been present in every species reported. The related *Pseudotrichonympha* occurs in all other genera of the Rhinotermitidae listed above as examined, though apparently not in every species. With it there are often associated *Holomastigotoides* and *Spirotrichonympha*.

Dunkerly (1929) has given an interesting discussion of the non-effectiveness of natural selection in the case of highly differentiated parasitic organisms, such as cestodes and hypermastigote flagellates, whose environment has been comparatively unvarying. He points to the evolution of hypermastigotes into many markedly distinguishable genera and species from a simple trichomonad polymastigote ancestor (or ancestors) in termites. We must now, since the discovery by Cleveland, Sanders and Hall (1931) of highly developed hypermastigotes in the wood-boring roach *Cryptocercus punctulatus,* push this evolution back to the ancestral protoblattid insects.

The wide and uneven distribution of *Trichonympha* in termites is in striking contrast to the situation in certain of the groups of polymastigote flagellates, which appear to have undergone their evolution in special groups of termites. Thus the Oxymonadidae, Devescovininae, and Calonymphidae are, so far as is known, restricted entirely to the Kalotermitinae, except for the primitive representatives of the last two groups reported from *Hodotermes* (*Anacanthotermes*) *murgabicus* by Bernstein (1928). The Pyrsonymphidae are known to occur only in *Reticulitermes*, and the Streblomastigidae only in *Termopsis*. These groups of polymastigotes appear to have evolved in limited groups of the Isoptera, but must have undergone little change for a long period of time.

Among the hypermastigotes known in termites, only *Pseudotrichonympha* seems to admit the possibility of close evolutionary relationship to *Trichonympha;* yet it is in some features quite different from *Trichonympha*. *Pseudotrichonympha* is restricted to various genera of the Rhinotermitidae, except for *Pseudotrichonympha pristina* reported by Cutler from *Archotermopsis wroughtoni*. On the basis of Cutler's description, however, it seems to the writer that *Pseudotrichonympha pristina* should be placed in a different genus.

The facts of comparative morphology and distribution of *Trichonympha* seem to show that comparatively little evolution has occurred in the genus during the phylogenetic development of Isoptera. It is probable that the flagellates were present in much their present form in the most primitive termites. Evidence for the correctness of this view has been adduced in the report by Cleveland, Sanders and Hall (1931) of the existence in the wood-boring roach of species of *Trichonympha* much like those of termites. This discovery indicates the probable existence of hypermastigotes of the genus *Trichonympha* in the primitive ancestors of roaches and termites.

Wheeler (1923), citing Holmgren, points out that termites "probably came off from the ancestral blattoid stem in late Paleozoic or early Mesozoic times." He also states that it is probable that "the termites, like the ants, reached their complete structural and social development in the late Cretaceous or early Tertiary and have since undergone very little modification." If this suggestion is correct, it seems likely that *Trichonympha* existed in the late Paleozoic, and that by the late Cretaceous or early Tertiary all the main types of polymastigotes and hypermastigotes peculiar to termites were in existence.

COMPARATIVE MORPHOLOGY OF THE GENUS TRICHONYMPHA

SHAPE AND SIZE

The shape of *Trichonympha* varies with the activity of the animal, the anterior portion turning from side to side and back upon the body, so that it becomes shorter and wider. Under certain conditions the posterior end may become more or less deeply concave. When the animals are normally extended, however, all species studied agree in general form. The length is from two to three times the width, which is greater in the middle, and the body tapers anteriorly and in variable degree posteriorly. The anterior portion is quite constant in form, under given conditions of activity, in the different species, while the posterior portion varies. Sometimes this is broadly rounded, while often it tapers more or less. Occasionally, as is often the case in *T. agilis*, it comes to a point. Under normal conditions it is not so globular as it often is in fixed material.

Though *Trichonympha* appears to be radially symmetrical, and in some cases is, it often exhibits a fundamentally laeotropically spiral symmetry. This is exhibited in the parabasal cords, characteristically in *T. campanula;* in the spiral arrangement of the basal rods and their connections in *T. campanula* and *T. collaris;* in the direction of the posterior flagella, and sometimes, especially in some specimens of *T. magna,* in the spiral direction of the ridges and plates. The laeotropically spiral direction of the ridges and flagella is shown by Grassi and Sandias (1893, pl. 5, fig. 4) in *T. agilis.* The spiral rows of flagella in *Pseudotrichonympha* are laeotropic (fig. D, 1, p. 416), so evidently this is a fundamental feature of the organization of the family Trichonymphidae.

The smallest species known are those of the *agilis* group, including, besides *T. agilis, T. minor* and the species from *Hodotermopsis.* The first species averages in length about 75μ, with a minimum of about 54μ and a maximum of about 115μ, and the others are similar in size. Larger in size are those belonging to what may be designated as the *chattoni* group, including the six species known from *Kalotermes,* and *T. sphaerica.* Those from *Kalotermes* agree closely in size, average length of four species being respectively 109, 111, 112, and 112μ, with a minimum of from 70 to 90μ, and a maximum of from 130 to 150μ. *T. sphaerica* is about 50 per cent larger than these. The largest species are those of the *magna* group, which includes *T. magna, T.*

campanula, T. turkestanica, and *T. collaris.* These average about from 150 to 250μ, with a minimum length of about 114μ (*T. magna*), and a maximum of about 360μ (*T. collaris*). The greatest dimensions recorded in the literature were for *T. campanula,* which reaches a length of 450μ, according to Kofoid and Swezy. The writer, however, has not encountered any specimens of this species greater in length than 312μ.

SUBDIVISIONS OF BODY

The body of *Trichonympha* is subdivided into three regions: the rostrum, at the anterior end of which is the cap; the flagella-bearing region of the body behind the rostrum; and the non-flagellated posterior portion.

The cap-like structure, which is entirely free of flagella, and which collapses readily under certain abnormal conditions, is characteristic of *Trichonympha* and *Pseudotrichonympha.* The term cap was originally applied to it by Porter (1897) and this has been accepted by most later investigators. Kofoid and Swezy named it operculum and De Mello (1927) used the term head for this structure in *Pseudotrichonympha belari.* The size of the cap varies with the size of the whole animal, from about 6 to 8μ in diameter at the base in *T. agilis* and the species from *Kalotermes* to from 17 to 20μ in *T. collaris,* but it is quite constant in a species. Especially interesting is the structure of this portion of the body in *T. turkestanica,* where there is a membrane within the outer membrane of the cap and outside of the granule in its base. This inner membrane has not been observed in other species from termites.

The cap is part of the rostrum, which was designated as the mammilla by Grassi and Sandias (1893), and the nipple by Porter. Duboscq and Grassé proposed the term rostrum, which has been adopted here. De Mello referred to this portion of the body in *Pseudotrichonympha belari* as the neck, but *Pseudotrichonympha* does not possess the rostral fissure. In this paper the outer portion of the rostrum has, because of its form, been referred to as the collar.

The dimensions of the rostrum are quite constant in a species. In length, taken from the point where the rostral tube joins the body to the anterior end of the cap, rostra of different species vary from 9.5 to 29μ. The peripheral margins of the collar are generally posterior to its innermost portion. Rostra of different species differ in proportions. Those of the species in *Kalotermes* are slender, the diameter at

the posterior end of the collar being only slightly greater than the length. In other species, however, this diameter is often about 50 per cent greater than the length, while in *T. agilis* and *T. sphaerica* it is almost twice the length.

The cleft or fissure between the rostrum and the rest of the body was observed and accurately figured by Porter in *T. agilis*. He gives a drawing of an animal which had been placed in diluted milk, in which the collar was elevated and separated from the following part of the body (his pl. 1, fig. 4), just as in *T. campanula* and *T. collaris*, as mentioned above. The cleft is a general characteristic of the genus *Trichonympha*. Grassi (1917) interpreted this ("cytartrosi") as a circular cavity filled with liquid and closed on the surface by a delicate membrane, and Kofoid and Swezy described it in the same way. Koidzumi, and Duboscq and Grassé recognized the fissure in its correct form, but Bernstein regarded it as a ring-formed vacuole.

The body behind the fissure consists of an anterior, flagella-bearing portion, with ridges and plates, and a posterior, non-flagellated portion. The former region was called the bell by Porter and by Koidzumi, since it has a campanulate or conical form. Its sides are straight or convex. The two regions of the body often are marked off from one another by a shoulder-like projection just beyond the posterior ends of the ridges. This is an especially prominent feature of *T. agilis*. In many cases this shoulder-like projection is limited to the contour of the endoplasm, and does not involve the outline of the body.

The ratio between the length of the flagellated and non-flagellated regions of *Trichonympha* differs in different species. Though varying some within a species, this ratio provides a good criterion of specific distinction. The flagellated region is least extensive in the species from *Kalotermes*, in which it averages about a third of the body length, and in *T. sphaerica*, in which it averages less than a third of the total length. The species of the *agilis* and *magna* groups have more extensive flagellated regions; in the former usually more than a third to almost a half and in the later usually more than a half. The most extensive flagellated region is in *T. campanula*, in which it occupies on the average nearly two-thirds of the length.

SURFACE RIDGES AND FLAGELLA

In all species of *Trichonympha* the flagella-bearing portions of the rostrum and body are furnished with rounded ridges, from the grooves between which the flagella emerge. The ridges on the rostrum are about half as numerous as those on the body. This latter number varies in different species. In *T. magna* and *T. chattoni* there appear to be between forty and fifty, in *T. agilis* about eighty, in *T. campanula*, *T. turkestanica*, and *T. collaris* about one hundred. When a normal *Trichonympha* is observed, the rostral ridges appear to be continuous with the ridges of the body, as they were stated to be by Kofoid and Swezy, who, however, were referring to the plates. Grassi (1917) was also unable to make out the interruptions between the ribs of the two regions, and believed them to be subdivided at the level of the cytarthrosis. Actually, however, they are interrupted by the rostral fissure.

Grassi (1917) accurately described and figured the rounded ridges in *Trichonympha magna* (his pl. 5, figs. 6, 13, 14). He defined them as projections of the body between the plates, which he called ribs or myonemes, and he observed them in some cases as tubules between the ribs.

Grassi noted that in mounted preparations there were cases in which, instead of the ridges protruding, they were depressed, so that the ribs appeared as crests from the summits of which the flagella emerged. This, which is doubtless an abnormal condition, is sometimes the case in preparations of *T. campanula* and *T. collaris*, and it gives an explanation of the failure of Porter, and of Kofoid and Swezy, to observe the surface ridges themselves.

The flagella of *Trichonympha* are exceedingly numerous. The writer has estimated that in *T. campanula* there are probably at least ten thousand, while in the species with more restricted flagellated regions there are probably at least six or seven hundred. Leidy believed that they arose in three or four circles, corresponding to the demarcations which appear on the surface between the different regions of the body. Actually, however, they are evenly distributed over the whole of the flagelliferous region. Their roots extend through the ectoplasm to its innermost layer, where are situated the basal granules.

The flagella of all species appear to be of relatively similar length. In all cases there are long posterior flagella extending along the sur-

face of the non-flagellated region of the body and beyond it to form a posterior fascicle, which, in *T. agilis* at least, may serve for attachment to objects and for entanglement of debris. The flagella of the rostrum of an individual appear to be all of similar length, except that in some cases there are a few short ones anteriorly. The flagella of the body posterior to the rostrum in many cases increase gradually in length to the longest terminal ones, but in *T. campanula, T. collaris,* and *T. magna* there is an extensive middle zone of flagella shorter than those on the rostrum.

ECTOPLASMIC REGION

The ectoplasm of the rostrum and anterior body region is thick in all species of *Trichonympha*, while that covering the posterior portion is thin. In some species, as for example in *T. campanula*, the ectoplasm of the anterior portion of the body tapers posteriorly, that just posterior to the rostral fissure being much thicker than that near the nuclear region. In others, as *T. sphaerica, T. agilis, T. lighti*, and *T. magna*, this does not so taper, but is just as thick in the nuclear region as in the rostrum. In these species, the endoplasm extends abruptly outward at the end of the flagellated zone.

There are three layers of ectoplasm: outer, middle, and inner. The outer layer, which contains the plates, is dense and is as thick or thicker than the other two combined. By careful observation, flagella can be observed traversing it.

What has been said above of the number and arrangement of the ridges applies also to the plates, the peripheral edges of which lie beneath the grooves between the ridges. They consist of dense stainable protoplasm, enclosing the roots of the flagella, and traversing the entire radial extent of the outer layer of ectoplasm. They are sometimes bent at their inner ends, as in *T. magna* (pl. 28, fig. 44).

The middle, clear layer varies some in relative thickness in different species, being especially thick in *T. magna*, where instead of decreasing in thickness posteriorly, as it usually does, it is thickest at its end. It is, in all cases, relatively thin in the rostrum. In living material the roots of the flagella can clearly be seen in this layer in the body region, but not in the rostrum. The middle layer is probably denser in the rostrum than in the body region, where it is fluid.

The clear middle layer of ectoplasm, traversed by the roots of the flagella, was accurately described and figured by Porter and by Grassi. Koidzumi believed that the flagella arose at the boundary between

this and the outer layer, as he failed to see the roots of the flagella in it. The boundary between the outer and middle layers is well defined, but there is no layer of granules here. This may be clearly ascertained in sections (pl. 21, fig. 5).

The inner layer is most conspicuous in the rostral region, where in all species it is of relatively similar thickness. It is usually thinner in the body region, where in some species, as in *T. agilis*, it can hardly be seen at all. In *T. sphaerica*, however, it is as thick here as in the rostrum, and does not taper off posteriorly, as it does in most species. The existence of this layer was first pointed out by Koidzumi, but it is indicated in some of Grassi's figures. Duboscq and Grassé described it in *T. chattoni*.

Across the anterior end of the ectoplasm of the body, in optical section, there appear dense, stainable bands which probably are optical sections of a disc. This structure appears to be characteristic of all species. In *T. agilis* indications of it were given by Grassi (1917) and by França (1916, 1918), as pointed out above in the account of that species (p. 410).

<center>BLEPHAROPLAST</center>

The hemispherical granule in the base of the cap is present in all species of *Trichonympha*. Most workers, however, have either overlooked it or described it inaccurately. In its basal portion is an especially deeply staining ring, or pair of granules, and only this portion of the blepharoplast was seen by Duboscq and Grassé in *T. chattoni* and probably by Kofoid and Swezy in *T. campanula*. Porter, Koidzumi and Grassi observed it inaccurately as merely an enlargement at the anterior end of the axial core or rostral tube, and not sharply demarcated from this. As stated in the above account of *T. agilis*, França (1916, 1918) described it and applied to it the term blepharoplast, which the writer has adopted. In the first paper, however, he included the rostral tube also under this term, but later he appears to have restricted it. The term blepharoplast has been adopted because to it the numerous flagella are ultimately connected and because it suggests a blepharoplast in position and method of division.

Koidzumi (1921) and De Mello (1927) have reported that in the division of *Pseudotrichonympha* a desmose appears between the divided granules at the anterior end of the rostral tube. Probably this is also the case in *Trichonympha*. Kofoid and Swezy have shown some indication of it in *T. campanula* (their pl. 8, fig. 34).

In a number of hypermastigotes other than *Trichonympha* and *Pseudotrichonympha* there are one or two granules, not included in the group of basal granules of the flagella but presumably ultimately attached to them, which have been termed blepharoplasts. In *Hoplonympha* (Light, 1926) the blepharoplast is "a granule or irregular siderophile mass" where the two flagellar plates meet anteriorly, and it lies in the center of the cavity of a bowl-shaped terminal cap. In *Staurojoenina* there is a quadripartite anterior siderophile structure, to which the peripheral bands, which constitute the flagellar plates and contain the small basal granules, are connected; this has been called (Kirby, 1926) the centroblepharoplast, but probably is better designated simply as the blepharoplast. The blepharoplast of *Joenia* is a small granule lying above the nucleus near the group of flagella and attached to a filament of the parabasal apparatus. In *Joennia* (Grassi, 1917) there are two curved rodlets, which together form a V, in a similar position. In division of *Joenia* the two division products of the blepharoplast come to be situated at the poles of the paradesmose (Grassi and Foa, 1904).

Cupp (1930) found a disc-shaped, deeply staining blepharoplast in *Spirotrichonympha polygyra* at the anterior end of the cone composed of the flagellar bands; from it the flagellar bands and the rhizoplast take origin. This also, like that of *Hoplonympha* and the Trichonymphidae, is situated under a cap or operculum. De Mello (1928) found a siderophile granule in the same position in *Spirotrichonympha* from *Leucotermes indicola*. In most species of *Spirotrichonympha* the flagellar bands are very closely wound anteriorly, forming a siderophile tubule comparable to the rostral tube of *Trichonympha*. This apparent tubule, however, cannot properly be called a centroblepharoplast, as was done by Brown (1930).

In *Cyclonympha* Dogiel (1917b) used the term blepharoplast for a siderophile structure at the anterior end of the axial column from which the head flagella take origin. Koidzumi (1921), in his more detailed study of the same flagellate (as *Teratonympha*), does not describe this structure, but it seems likely that the inner cone of the axial column, which is situated anteriorly and is said to stain especially deeply, is the blepharoplast described by Dogiel.

This brief survey of conditions in some other members of the order Hypermastigida is sufficient to show that in the comparative morphology of that group of flagellates there is support for the nomenclature adopted in this paper for the granule surmounting the rostral tube of *Trichonympha*.

ROSTRAL TUBE AND BASAL BODIES OF FLAGELLA

The rostral tube has been called by various names, but its presence has in almost all accounts been clearly indicated in *Trichonympha*. Grassi and Sandias (1893) wrote of it as a cylindrical tube, and Grassi (1917) as a tube, filled with a colorless liquid, which ends anteriorly in a rounded cap. Porter named it the axial rod, which he described as expanding anteriorly into a knob. França, who appears to have believed it to be solid, referred to it (1916) as a stem joining the two portions of the body and (1918) designated it and the blepharoplast as the parabasal body and blepharoplast. Koidzumi (1921) used for it the term axial core. The nomenclature used here has been adopted from Duboscq and Grassé (1927).

Kofoid and Swezy used for the rostral tube, including those parts of the anterior granule which they observed, the term centroblepharo-plast, and De Mello adopted the same terminology for *Pseudotricho-nympha belari*. The rostral tube, however, probably consists of fused basal granules, and is not to be differentiated in nature from the rest of the layer of basal granules. It follows from the definition of cen-troblepharoplast that the paradesmose would connect the two parts resulting from division at the time of mitosis. While sometimes, as reported, the paradesmose in *Trichonympha* may connect the bases of the rostral tubes, this is not the case, at least, in *T. chattoni* and *Pseudotrichonympha*. Consequently the writer has not found it possible to follow the terminology used for this structure in *T. campanula*.

In all species of *Trichonympha* which the writer has studied the rostral tube has a solid deeply stainable wall.

Basal granules of the flagella of *Trichonympha agilis* were shown by Porter (1897) and by Grassi (1917) in the innermost layer of the ectoplasm. Koidzumi, who believed that the flagella arise from between the layers designated in this paper as outer and middle, did not observe the basal granules. Kofoid and Swezy did not mention the basal rods described in this paper in *T. campanula,* but they placed the basal granules deep in the endoplasm, just above the so-called oblique fibers with which the granules are said to be con-nected by branches. In *Trichonympha turkestanica* the structures called basal granules by Bernstein are not such granules, but probably peripheral granules. Duboscq and Grassé correctly placed the basal granules at the innermost layer of the ectoplasm.

The basal bodies in *T. campanula* and *T. collaris* are unusual in being rodlets, while in *T. agilis* and *T. magna* they are granules. In all cases they are situated in the deepest portion of the ectoplasm. In *T. magna* the granules appear to be connected together in circular arrangement, and just posterior to the rostral tube they are so closely approximated as to constitute rings. In *T. campanula* and *T. collaris* the connections are differently arranged; the rodlets are situated obliquely in laeotropic spirals, and are connected by their ends along the spirals and also longitudinally at one end. Thus the whole system of basal bodies is interconnected, and ultimately joins the blepharoplast. The connections have not been studied so closely in other species as in these.

MYONEMES

Grassi (1917), describing the plates in *Trichonympha agilis, T. minor*, and *T. magna*, called them myonemes or ribs. He did not describe their true radial thickness, but suggested the possibility that they represented folds in the pellicle. The plates have been referred to by others as myonemes, and it is probable that all descriptions of longitudinal myonemes in *Trichonympha*, except those of Kofoid and Swezy, have reference to them. The longitudinal myonemes described by Kofoid and Swezy in the outer portion of the endoplasm of *T. campanula* possibly were the parabasal cords, as pointed out by Duboscq and Grassé. It has not been possible for the writer to trace the parabasal cords to the base of the rostrum in this species, while Kofoid and Swezy's longitudinal myonemes are shown clearly all the way. But no other filamentous structures in the outer layer of the endoplasm of *T. campanula* have been observed by the writer.

Circular transverse myonemes were described by Kofoid and Swezy in the innermost layer of ectoplasm, just beneath what has been designated here as the middle layer. Their position apparently corresponds, approximately at least, to that of the basal rods. As stated above (p. 364), the writer has observed, in whole mounts, circular or nearly circular striations corresponding in interval to their descriptions of myonemes. It has seemed to him, however, that these striations are optical illusions, and are due to the arrangement of the basal rods and the deeper portions of the flagellar roots, the former of which were not observed by Kofoid and Swezy. No trace of the so-called myonemes has been seen by the writer in sections.

As pointed out above (p. 370), it seems possible to account for all of the body movements of *Trichonympha* without assuming the existence of myonemes.

PARABASAL APPARATUS

A parabasal apparatus of the type described in the specific accounts in this paper is probably a general characteristic of the genus *Trichonympha*. Grassi and Sandias (1893) and Grassi (1917) described in *T. agilis* a little basket, in the bottom of which the nucleus was situated, composed of curved rods of dense protoplasm which were resistant to acetic acid. Traces of it were discerned by Porter (1897) in the same species, and were described as "a granular protoplasmic layer separating the 'bell' from the posterior part" of the body, forming a bowl-shaped structure within which the nucleus was located. Foa (1904) described the basket-like structure and showed the cords during division stages. She stated that the apparatus, which serves to contain the nucleus, is comparable to the collar of *Joenia,* which, as we now believe, is the parabasal apparatus. Koidzumi, like Porter, was unable to see the apparatus clearly, but believed that a membranous sac suspended the nucleus. As mentioned above, Kofoid and Swezy, who must have seen portions of the parabasal cords in living material, described them as longitudinal myonemes. Duboscq and Grassé were the first to give an accurate account of the apparatus in *Trichonympha,* and to state clearly its parabasal homologies, which had been suggested by Janicki (1915).

The acceptability of the terminology of Duboscq and Grassé will, no doubt, seem doubtful to the reader. The multiplicity of the cords does not present great difficulty; a multiple parabasal apparatus associated with a single nucleus is present in other flagellates. It may, however, seem that the cords should rather be homologized with the body filaments present in some other hypermastigotes, or with the axostylar apparatus. But they are quite unlike these structures in staining reactions, in which they do resemble the parabasal apparatus such as that of *Devescovina.* Furthermore, in detailed structure the cords resemble parabasal bodies of trichomonads and devescovinids.

The situation in *T. agilis* has misled protozoologists into regarding the structure as primarily a nuclear suspensory apparatus. In what are probably the more primitive species, as some of those in the wood-boring roach, and *Trichonympha chattoni,* the cords do not come into contact with the nucleus, except occasionally. There is no evidence in

any case that the cords are firmly attached to the membrane, except that occasionally the nucleus is distorted in a way that might be accounted for by the pulling of the cords. This, however, need not be interpreted as due to that cause.

There is considerable variation in the arrangement of the parabasal apparatus in different species of *Trichonympha*. It is, in fact, one of the most useful specific characteristics. The principal types of arrangement are these: that in which the cords are separate and ordinarily free from contact with the nucleus (*Trichonympha* from *Cryptocercus punctulatus, T. chattoni, T. sphaerica*); that in which some of the cords are free and some, after contact with the nucleus, extend posterior to it (*T. campanula*); that in which all of the cords ordinarily come into contact with the nucleus and extend posterior to it (*T. collaris, T. magna*); that in which the cords are in cylindrical arrangement, close to one another, around the nucleus and posterior to it (*T. saepiculae*); and that in which there is more or less of a basket arrangement, with the posterior ends of the cords usually not extending far beyond the nucleus (*T. lighti, T. quasilli, T. agilis*).

In a species of *Pseudotrichonympha* from *Leucotermes aureus* Snyder of southern California, a parabasal apparatus like that of *Trichonympha* has been discovered by the writer (fig. D, 2, p. 416). It has not been recognized in the genus heretofore, as it cannot easily be found in specimens prepared by the usual Schaudinn-iron-haematoxylin technique. In preparations made by the Champy-Kull method a number of cords extending from the posterior portion of the region of thick ectoplasm to the vicinity of the nucleus were found. Their arrangement is similar to that in *Trichonympha lighti*, but in some specimens some cords extended posterior to the nucleus.

NUCLEUS

The range in diameter of the nuclei of the various species of *Trichonympha* described in this paper is from 6μ in *T. lighti* to 35μ in *T. collaris*. The ratio between the size of the nucleus and the size of the body is not the same in all species, though the large species have large nuclei. *T. agilis*, with an average length of only about 76μ, has a nucleus of about 12μ, while in *T. lighti*, whose average length is 113μ, the nuclear diameter is only about 7μ. The nucleus of *T. saepiculae* is twice the size of the nucleus of *T. lighti*, although the body size of the two species is about the same.

The nucleus is always situated near the region between the flagellated and non-flagellated portions of the body, so that its position varies with the ratio between these regions. In some species, the nucleus is located entirely within the posterior part of the flagellated region (*T. collaris, T. campanula*), while in others it is entirely in the anterior portion of the non-flagellated region (*T. magna*). In many cases its position is where the two meet.

In internal structure the nuclei of various species also do not agree, so that the size, position, and structure of the nucleus are valuable systematic characters. The figures on plate 30 are intended for comparison of the nuclei of the various species in size and internal structure. All of these were drawn from nuclei of form characteristic for the species, and are believed to represent nuclei typical for the species and not reorganized prior to or subsequent to mitosis. By this it is not meant to imply that there is not considerable variation in the distribution of the chromatin in any given species; the descriptions pertain to the majority of nuclei encountered in the preparations.

The chromatin is in some species dispersed into separate granules, which may be in linear series or groups (*T. agilis, T. tabogae*), but in most this seems not to be the case. In *T. lighti, T. quasilli,* and *T. chattoni* the chromatin is in rods of variable length, or grouped granules. In *T. saepiculae* and *T. subquasilli,* among the species from *Kalotermes,* and in all the species from *Termopsis,* coiled strands are present in almost all nuclei. The chromatin in each of these appears to be permanently organized into a spireme, complete or segmented. The strands are considerably stouter in *T. collaris* than in *T. campanula.* Strands which, in comparison with those in nuclei of other species, are unusually stout are present in *T. magna.*

In some cases there is normally a wider chromatin-free space beneath the membrane than in other species, but the chromatin is never, under normal resting conditions, contracted far from the membrane. In *T. magna* there is usually no such space at all.

In addition to the chromatin there is usually present a small, peripherally situated body in a clear space. This has not been seen in *T. collaris, T. sphaerica,* or *T. lighti,* but is present in all other species described in this paper. Duboscq and Grassé, describing it in *T. chattoni,* called it a nucleolus or endosome; Bernstein, in *T. turkestanica,* called it Binnenkörper; and Kofoid and Swezy, in *T. campanula,* heterochromosome.

This body is in all cases situated just beneath the nuclear membrane within a clear space, around which there is no evidence of a membrane. It is spheroidal in form in all species reported except *T. campanula,* in which it is generally a short, curved, bent or coiled rodlet, though sometimes this is broken into parts. It varies in size in different specimens of a species, and appears to be especially large in some species, as *T. chattoni* and *T. campanula.*

In *T. campanula* this body stains like the chromatin with all the dyes used, including the Feulgen reaction. According to Bernstein, however, the similar but spherical body in *T. turkestanica* does not stain with the Feulgen reaction. This test for chromatin has not been used for other species. In all cases the body stains intensely with iron-haematoxylin, but in some it was unstained in preparations where the chromatin was stained with Delafield's haematoxylin.

The data concerning the microchemical nature of this body and its behavior during the division process are insufficient to warrant a conclusive statement as to its homologies. In *T. campanula,* according to Kofoid and Swezy, it persists throughout mitosis, dividing into two somewhat later than the chromosomes. In *T. chattoni* it apparently behaves in a similar manner, according to Duboscq and Grassé.

ENDOPLASMIC INCLUSIONS

The endoplasmic inclusions, exclusive of parasites, described in this paper are the minute endoplasmic granules, the anterior endoplasmic granules, the chondriosomes, the neutral red staining granules and spherules, the bodies staining with both neutral red and haematoxylin, and the ingested food material.

The minute endoplasmic granules are glycogen-like, staining with iodine. Buscalioni and Comes (1910) stated that in *T. agilis* the glycogen, partly in the form of minute granules, partly diffuse, was located in the rounded, bottle-shaped region of endoplasm ("bottiglia") anterior to the nucleus. The posterior part of the body, which they believed to be separated from this part by a membrane, took only the yellowish color characteristic of protoplasm. In *Trichonympha campanula,* however, the iodine-staining granules are present also in the posterior endoplasm, though they are not so closely packed. Buscalioni and Comes stated that the localization of the glycogen reaction to the part anterior to the nucleus gave a condition comparable to the functional existence of a minute hepatic gland. Grassi

(1917) denied that the small granules described by him were glycogen, as Buscalioni and Comes claimed, since they were preserved by methods not suitable for glycogen. There are many granules, however, besides the minute, iodine-staining endoplasmic granules, as described in the next paragraph. Some of these stain with haematoxylin; doubtless Grassi had reference to them.

The anterior endoplasmic granules are present in all species, but they differ in form, distribution, and size. There are at least two categories of granules under consideration. Some stain readily with haematoxylin, while others give reactions more like those of lipoidal bodies or chondriosomes. Anterior endoplasmic granules similar in form and distribution to those of *Trichonympha* have been observed in *Pseudotrichonympha* sp. from *Leucotermes aureus* (fig. D, 2).

The chondriosomes, situated in the postnuclear endoplasm, resemble the typical rod-like structures given this name elsewhere. They are, at least in the species from *Termopsis*, demonstrated well after use of most of the usual mitochondrial methods; however, the writer has not been able to stain them with Janus green B. In some cases bodies similar in form are shown to be present after use of Schaudinn's fluid with acetic acid, but these probably belong in a different category. That those so named above are really chondriosomes is not certain, but they resemble chondriosomes so closely that the writer has seen no reason for changing Duboscq and Grassé's terminology used for them in *T. chattoni*, in which they were first observed.

The genus *Trichonympha* is divided into two groups with respect to the neutral red staining granules and spherules. In *T. sphaerica* and all species from *Kalotermes* there are large and conspicuous spherules. In *T. campanula*, *T. collaris*, and *T. agilis* there are only a few small granules which take the stain. There is some variation in size of these bodies in different individuals of the same species, but not so much as between the two groups of species.

The bodies staining with both neutral red and haematoxylin are probably residues of food digestion. These have been observed in *T. collaris* and *T. chattoni*, but not in all species. Perhaps their presence is correlated with the digestion of microorganisms, ingestion of which seems to be more common in *T. collaris* and *T. chattoni* than in other species. Wood is ingested as the principal food by all species of *Trichonympha*. It is taken in through the posterior end of the body, probably mainly in the manner described by Cleveland (1925a).

ENTOZOIC MICROORGANISMS

The flagellates of termites are frequently parasitized by other organisms. The manner in which they live, and the ease with which organisms are passed from termite to termite, render these Protozoa highly favorable hosts for invading organisms. Many different kinds of entozoic microorganisms occur in both polymastigotes and hypermastigotes. The incidence of some of them is high. In this paper ten are reported from *Trichonympha*. Most of these occur in the species in *Termopsis*, which have been most carefully explored for parasites.

The peripheral granules situated in the outer layer of the anterior ectoplasm of *T. collaris* and *T. turkestanica* are not certainly parasites. The fact that they are absent from most species of *Trichonympha* makes it seem unlikely, however, that they are normal structures of the flagellates. Their presence in uniform abundance in all individuals of *T. collaris* is especially interesting if they are parasites.

The proximo-nuclear bacilliform or filamentous parasite of *T. campanula* and *T. collaris* is also of especially interesting incidence, being present in all specimens, in approximately uniform abundance.

The parasites are of diverse natures, and their true relationships have not for the most part been established. An unusual one has been encountered in the endoplasm of *T. magna* (p. 395), and an interesting intranuclear parasite, which probably is not *Nucleophaga*, is present sometimes in *T. saepiculae* (p. 405). *Sphaerita*, which occurs in several species, has been encountered most frequently in *T. agilis* (p. 413). *T. agilis* sometimes has also a bacilliform parasite.

KEY TO THE KNOWN SPECIES OF TRICHONYMPHA

A key is apt to be of temporary value only, and may be rendered obsolete when new facts are discovered. That a key may be based on entirely erroneous distinctions if its author does not have complete, first-hand knowledge of the organisms concerned is illustrated by the keys given by Calkins (1926) to the Trichonymphidae and by Bernstein (1928) to the genus *Trichonympha*. The writer has attempted to prepare a key for *Trichonympha*, partly in order to correct the errors in these, and partly to point out some of the distinctions

between species. For known species, however, a knowledge of the host termite will be of more use, and for unknown species a careful comparison with all characteristics of described forms is necessary; hence, it is not expected that this key will be of very much practical value.

Calkins' key to the family Trichonymphidae is based on the arrangement and length of the flagella. *Trichonympha* is stated to have these in three zones; that is true only of a few species, and not of the type species nor of most others. *Pseudotrichonympha*, by the diagnosis of "flagella in two zones," could not be separated from *Trichonympha* in some cases. *Leidyonella*, which is included in the key, is not a recognized genus, and *Leidyopsis* (*Trichonympha sphaerica*), said to have relatively short flagella, does not have shorter flagella than the other species of *Trichonympha*.

Bernstein's key is based on the "suspensorial apparatus," the arrangement and length of flagella, and the dimensions of the body. It is accurate so far as knowledge went at that time, but is inaccurate and incomplete on the basis of the findings in this paper. The parabasal apparatus is probably present in all species.

It is not possible to distinguish some species of *Trichonympha* from one another without suitable stained preparations.

1. Flagellated region typically occupying half or more of the length of the body. Size large.   2
   Flagellated region typically occupying about a third or less of the length of the body. Size moderate.   5
2. Most of the cords of the parabasal apparatus not touching the nucleus. Host: *Termopsis*. Length 144–333μ.   **T. campanula**
   All cords of parabasal apparatus passing close to or touching nucleus, or structure unknown.   3
3. Granular, cylindrical column present in center of prenuclear region. Peripheral granules absent. Host: *Porotermes*. Length 114–172μ.   **T. magna**
   Granular column absent. Peripheral granules present.   4
4. Rostrum large, length in center 26–29μ. Parabasal apparatus well developed, cords extending posterior to nucleus. Host: *Termopsis*. Length 168–360μ.   **T. collaris**
   Rostrum smaller, length in center 13–15μ. Parabasal apparatus unknown. Host: *Hodotermes*. Length 120–264μ.   **T. turkestanica**
5. Spherules stainable with neutral red not present, only a few granules taking the dye. Host: *Reticulitermes*.   6
   Spherules stainable with neutral red at least in some species, conspicuous in postnuclear endoplasm.   7
6. Parabasal apparatus basket-like, all cords meeting just behind nucleus. Length 55–115μ.   **T. agilis**
   Parabasal apparatus of separate cords extending posterior to nucleus and not all touching it. Length a little less than that of *T. agilis*.   **T. minor**

7. Parabasal apparatus of separate cords mostly free from contact with the
   nucleus.   8
   Parabasal apparatus associated with nucleus, basket-like or cylindrical. 10
8. Size large, rostrum broad, diameter at base of collar 24–26μ. Host:
   Termopsis. Length 108–215μ.   **T. sphaerica**
   Size smaller, rostrum narrower, diameter at base of collar about 13μ. Host:
   Kalotermes.   9
9. Chromatin of nucleus in solid granules, rods, or masses. Length 84–132μ.
                                                                      T. chattoni
   Chromatin of nucleus in small vesicles in linear series or groups. Length
   same.   **T. tabogae**
10. Parabasal cords all close together, forming a cylinder around and extending
    posterior to nucleus. Length 72–149μ.   **T. saepiculae**
    Parabasal cords mostly touching nucleus at posterior ends, forming more or
    less basket-like apparatus.   11
11. Nucleus small, 6–8μ in diameter. Length 94–138μ   **T. lighti**
    Nucleus larger, 8–13μ in diameter.   12
12. Parabasal apparatus regularly basket-like. Chromatin in small vesicles or
    grouped granules. Length 81–144μ.   **T. quasilli**
    Parabasal apparatus of sinuous cords and not so regularly basket-like.
    Chromatin in more of a continuous reticulum.   **T. subquasilli**

DIAGNOSES OF GENUS AND SPECIES

Trichonympha Leidy, 1877

?Leidyonella Frenzel, 1891.
Gymnonympha Dobell, 1910.
Leidyopsis Kofoid and Swezy, 1919.
not *Trichonympha* Kent, 1885.
not *Trichonympha* de Mello, 1920.

Relatively large hypermastigote flagellates, 54–360μ long; length
two to three times the width; tapering anteriorly and usually pos-
teriorly; radially symmetrical, or in certain features laeotropically
spiralled; body subdivided into three regions, rostrum with anterior
cap, flagella-bearing region behind rostrum, and non-flagellated pos-
terior region; first two of these regions, except cap, covered with
flagella in longitudinal rows; flagellated region varying in extent from
less than a third to more than two-thirds of length; flagella arising at
posterior limit of this region longest, extending beyond end of body;
rostrum separated by fissure extending inward to rostral tube; about
40–100 longitudinal surface ridges on body, half as many on rostrum;
flagella emerging between the ridges; thick anterior ectoplasm in three
layers, outer with plates, middle which is fluid in body region, and inner
which is usually thin in body region; ectoplasm traversed by roots of
flagella to basal granules in innermost layer; in rostrum basal gran-
ules fused into rostral tube; blepharoplast a hemispherical granule at
anterior end of rostral tube in base of cap; parabasal apparatus com-
posed of numerous cords in endoplasm of anterior region, often touch-

ing or forming basket around nucleus, sometimes free from it; nucleus single, near region between flagellated and non-flagellated parts of body; chromatin in granules, masses or spireme, nucleolus usually present; chondriosomes and various kinds of granules present in endoplasm; wood ingested by all known species; described species entozoic in termites.

Genotype.—Trichonympha agilis Leidy, 1877.

Trichonympha agilis Leidy, 1877

Trichonympha agilis var. *japonica* Koidzumi, 1916.
Trichonympha agilis var. *formosana* Koidzumi, 1916.
not *Trichonympha agilis* de Mello, 1919, etc.
Trichonympha serbica Georgevitch, 1930.

Dimensions of body: length 55–115μ, averaging about 76μ, width 22–45μ, averaging about 32μ; dimensions of rostrum: length in center 9.5–12μ, diameter at base of cap 7–8.5μ, diameter at base of collar 18–21μ; ratio of flagellated to non-flagellated region: 0.44:1.00 to 0.81:1.00, averaging about 0.64:1.00; flagella: most of uniform length of about 25–30μ on rostrum, others increasing in length to long ones extending in "a [laeotropically] twisted fasciculus with divergent ends" beyond the end of the body; ectoplasm of flagellated region ending abruptly posteriorly, without taper, inner layer on body very thin, middle layer moderately thick; plates in outer layer of body about 82; peripheral granules absent; parabasal apparatus of numerous slender, separate cords appearing in peripheral endoplasm at posterior end of flagellated zone and usually forming a bowl-shaped structure embracing the posterior part of the nucleus, occasionally extending beyond nucleus; nucleus somewhat anterior to middle of body, diameter 10–14μ, with small peripheral nucleolus and chromatin in discontinuous granules or masses; anterior endoplasmic inclusions: numerous small iron-haematoxylin staining granules closely packed; posterior endoplasmic inclusions: similar more scattered granules, smaller granules, a few minute neutral red staining granules, wood, etc.

Type host.—Reticulitermes flavipes Kollar. Eastern United States.
Additional hosts.—R. lucifugus Rossi. Europe. *R. speratus* Kolbe. Japan. *R. flaviceps* Oshima. Formosa. *R. hesperus* Banks. California. *R. tibialis* Banks. California.

Trichonympha minor Grassi and Foa, 1911

Similar to *T. agilis,* but a little smaller; flagella-bearing zone shorter; nucleus smaller and closer to anterior end; more closely approximated plates; parabasal apparatus distinctive, consisting of numerous separate cords which extend with a laeotropic slant beyond the nucleus, and not all touching nuclear membrane as is characteristic of the cords in *T. agilis.*

Type host.—Reticulitermes lucifugus Rossi. Europe.

Trichonympha campanula Kofoid and Swezy, 1919, emended

Trichonympha campanula Kofoid and Swezy, 1919, *partim.*

Dimensions of body: length 144–313μ, averaging 217μ, width 57–144μ, averaging 85μ, average ratio of length to width 2.85:1.00; dimensions of rostrum: length in center 21–26μ, diameter at base of cap 12–14 (19)μ, diameter at base of collar 33–36μ; ratio of flagellated to non-flagellated region: 1.30:1.00 to 2.50:1.00, averaging 1.80:1.00; flagella: in three zones, longer ones (30–60μ) on rostrum, a uniform set of shorter ones (20–25μ) on anterior portion of body, and a posterior zone of flagella gradually increasing in length to long ones extending beyond the end of the body; ectoplasm of flagellated region tapering posteriorly, with well defined middle layer, inner layer on body thin and soon tapering off; plates in outer layer about 97–112; roots of flagella attached to small interconnected rods in inner layer; peripheral granules absent; parabasal apparatus of numerous slender separate cords distributed in region between nucleus and ectoplasm, some touching nuclear membrane, extending a short distance beyond nucleus; nucleus situated near middle of body; diameter about 25 (18–33μ), with chromatin in rather slender coiled strands and characteristic elongated nucleolus or "heterochromosome"; prenuclear endoplasmic inclusions: small, irregular bodies or clusters of granules situated peripherally and near base of rostral tube, not iron-haematoxylin staining after ordinary fixatives; postnuclear endoplasmic inclusions: numerous small granules staining with neutral red, chondriosomes, and wood.

Type host.—Termopsis angusticollis Hagen. California.

Additional hosts.—Termopsis nevadensis Hagen. California. *Termopsis laticeps* Banks. Arizona.

Trichonympha collaris sp. nov.

Trichonympha campanula Kofoid and Swezy, 1919, *partim.*

Dimensions of body: length 168–360μ, averaging 247μ, width 72–168μ, averaging 114μ, average ratio of length to width 2.17:1.00; dimensions of rostrum: length in center 26–29μ, diameter at base of cap 17–20μ, diameter at base of collar 39–41μ; ratio of flagellated to non-flagellated region 0.74:1.00 to 2.14:1.00, averaging 1.32:1.00; flagella like those of *T. campanula;* ectoplasm of flagellated region as in *T. campanula,* but inner layer on body somewhat thicker, though tapering; number of plates in outer layer as in *T. campanula;* basal granules in inner layer small rods as in *T. campanula;* peripheral granules present in outer layer of ectoplasm both of body and rostrum; parabasal apparatus of numerous cords in groups of about 2–7, most touching or approaching close to nucleus and extending farther than in other species into postnuclear endoplasm; nucleus usually situated just anterior to middle of body, diameter about 30 (26×26–30×35)μ, with chromatin in rather stout coiled strands and no conspicuous nucleolus; prenuclear endoplasmic inclusions: small,

isolated granules not staining after ordinary fixatives with iron-haematoxylin, and many other granules which do so stain; postnuclear endoplasmic inclusions: some small granules staining with neutral red, frequently larger irregular bodies staining with neutral red and also iron-haematoxylin, chondriosomes, and wood; apparently ingests other organisms more commonly than do the other species in *Termopsis*.

Type host.—Termopsis angusticollis Hagen. California.

Additional host.—Termopsis nevadensis Hagen. California.

Trichonympha turkestanica Bernstein, 1928

Dimensions of body: length 120–264μ, width 43–115μ, rather slender in form; dimensions of rostrum: length in center 13–15μ, diameter at base of cap 11μ, diameter at base of collar 22–24μ; ratio of flagellated to non-flagellated region similar to that of *T. campanula;* flagella on rostrum and body of equal length, long flagella posteriorly; ectoplasmic layers as in *T. collaris*, but middle layer somewhat narrower; peripheral granules present in outer layer; parabasal apparatus unknown; nucleus situated in middle of body, relatively somewhat larger than in *T. campanula;* prenuclear endoplasmic inclusions: many small, spherical granules, stainable with iron-haematoxylin.

Type host.—Hodotermes (*Anacanthotermes*) *murgabicus* Vasiljev. Turkestan.

Additional host.—Hodotermes (*Anacanthotermes*) *macrocephalus* Desneux. India.

Trichonympha magna Grassi, 1917

Dimensions of body: length 114–172μ, width 42–60μ; dimensions of rostrum: length in center 12–13μ, diameter at base of cap 6–8μ, diameter at base of collar 16–20μ; length of flagellated region from half to two-thirds of length of body; flagella: in three zones, as in *T. campanula*, fairly long ones on rostrum, shorter ones on anterior portion of body, and posterior ones gradually increasing in length to long ones extending beyond end of body; ectoplasm not decreasing in thickness posteriorly, middle layer thick, broadening posteriorly and ending abruptly, inner layer as in *T. collaris;* number of plates in outer layer about 40–46; basal granules are small granules arranged in transverse rings, conspicuous in anterior portion of body only; parabasal apparatus of numerous (about 40–45) cords arising near posterior end of thick ectoplasm, situated in one layer close to one another, lying adjacent to, but not attached to the nuclear membrane, extending a short distance posterior to nucleus; nucleus usually posterior to the middle of the body, diameter 13–20, averaging about 17μ, with chromatin in stout coiled strands filling space within membrane; prenuclear endoplasmic inclusions: minute scattered granules and a finely granular, cylindrical column, 2–3μ in diameter, usually reaching from base of rostrum to nucleus; postnuclear endoplasmic inclusions: wood, small granules.

Type host.—Porotermes adamsoni (Froggatt). Australia.

Additional host.—Porotermes grandis Holmgren. Victoria, Australia.

Trichonympha sphaerica (Kofoid and Swezy, 1919) Duboscq and
Grassé, 1927

Leidyopsis sphaerica Kofoid and Swezy, 1919.
Trichonympha (*Leidyopsis*) *sphaerica* (Kofoid and Swezy) Duboscq and
Grassé, 1927.

Dimensions of body: length 108–215μ, averaging 165μ, width
70–132μ, averaging 89μ, average ratio of length to width 1.85:1.00;
dimensions of rostrum: length in center 14–15μ, diameter at base of
cap 10–12μ, diameter at base of collar 24–26μ; ratio of flagellated to
non-flagellated region: 0.30:1.00 to 0.50:1.00, averaging 0.40:1.00;
flagella increasing gradually in length, the posterior ones being longer
than the body; ectoplasm of flagellated region of even thickness to
posterior end, where there is an abrupt shoulder of endoplasm, inner
layer of even thickness throughout; peripheral granules absent; para-
basal apparatus of numerous separate cords distributed evenly in
region around nucleus, some touching membrane, extending a short
distance beyond nucleus; nucleus situated anteriorly, near posterior
end of flagellated zone, diameter about 23 (19–30)μ, with chromatin
in coiled strands, and no conspicuous nucleolus; prenuclear endo-
plasmic inclusions: small, spherical granules not iron-haematoxylin
staining after ordinary fixatives, and some small granules which do so
stain; postnuclear endoplasmic inclusions: numerous neutral red
staining spherules, 1–7 (averaging about 4)μ in diameter, some
neutral red staining granules, chondriosomes, and wood.

Type host.—Termopsis angusticollis Hagen. California.
Additional host.—Termopsis nevadensis Hagen. California.

Trichonympha chattoni Duboscq and Grassé, 1927

Dimensions of body: length 84–132μ, averaging 109μ; width
36–57μ, averaging 46μ (or, according to Duboscq and Grassé, length
60–180μ, width 45–50μ in specimens of length 115–125μ); dimensions
of rostrum: length in center about 12μ, diameter at base of cap about
6μ, diameter at base of collar about 13μ; flagellated region occupying
from somewhat less to somewhat more than a third of the body length;
flagella increasing gradually in length from anterior ones on rostrum
to posterior ones which extend beyond the end of the body; middle
layer of ectoplasm unusually thin, difficult in some material to dis-
tinguish from inner layer; parabasal apparatus of numerous (32–44)
slender cords, rather straight, most not touching nucleus, and reaching
a short distance posterior to it; nucleus at about one-third of distance
from anterior end, diameter about 10 (6–13)μ, with chromatin in
granules, rods, or threads, and nucleolus of moderate size; prenuclear
endoplasmic inclusions: numerous small granules; postnuclear endo-
plasmic inclusions: spherules 1–5μ in diameter as in *T. sphaerica*,
chondriosome-like threads and granules, large spherical bodies stain-
able with iron-haematoxylin, wood, and ingested organisms; parades-
mose not connecting bases of rostral tubes.

Type host.—Kalotermes (Glyptotermes) iridipennis Froggatt. Australia.

Additional host.—Kalotermes (Kalotermes) contracticornis Snyder. Costa Rica.

Trichonympha tabogae sp. nov.

Similar to *T. chattoni* in most respects. Differs in (1) arrangement of chromatin in small vesicles in linear series or groups, with small peripheral nucleolus, instead of in solid granules, rods, or masses with larger nucleolus; (2) large characteristic posterior endoplasmic spherules and iron-haematoxylin staining spherical bodies of *T. chattoni* replaced by numerous smaller granules and spherules. Parabasal apparatus obscure in material studied, apparently like that of *T. chattoni*.

Host.—Kalotermes (Kalotermes) tabogae Snyder. Panama.

Trichonympha quasilli sp. nov.

Dimensions of body: length 81–144μ, averaging 111μ, width 31–53μ, averaging 38μ; dimensions of rostrum: length in center 9.5–11μ, diameter at base of cap 6–7μ, diameter at base of collar 12–14μ; ratio of flagellated to non-flagellated region: 0.29:1.00 to 0.62:1.00, averaging 0.44:1.00; flagella as in *T. chattoni;* middle layer of ectoplasm thicker than in *T. chattoni;* parabasal apparatus of numerous cords meeting behind the nucleus to form a little basket, in the bottom of which the nucleus is situated; nucleus at about one-third of the distance back from anterior end, 8–13μ in diameter, with numerous small isolated or grouped chromatin granules and a nucleolus of moderate size; prenuclear endoplasmic inclusions: numerous small granules; postnuclear endoplasmic inclusions: numerous spherules as in *T. chattoni*.

Host.—Kalotermes (Kalotermes) snyderi Light. Costa Rica.

Trichonympha subquasilli sp. nov.

Like *T. quasilli* except in the parabasal apparatus and the structure of the nucleus. The parabasal cords mostly converge toward the posterior part of the nucleus, but are more sinuous, some are free, and there is not so regular a basket as in *T. quasilli*. The chromatin in the nucleus is in more of a continuous reticulum instead of isolated or grouped granules.

Host.—Kalotermes (Kalotermes) clevelandi Snyder. Panama.

Trichonympha lighti sp. nov.

Dimensions of body: length 94–138μ, averaging 113μ, width 36–65μ, averaging 52μ; dimensions of rostrum: length in center 11–12μ, diameter at base of cap 6–8μ, diameter at base of collar 11–13μ; ratio of flagellated to non-flagellated region: 0.36:1.00 to 0.50:1.00, averaging 0.42:1.00; flagella as in *T. chattoni;* ectoplasm of flagella-bearing region of uniform thickness, with abrupt termination, as in *T. sphaerica;* parabasal apparatus of numerous (30–40) very slender, sinuous cords ending freely beside or anterior to nucleus, or in most cases touching anterior portion of nuclear membrane; nucleus within posterior limits of flagellated zone, 6–8μ in diameter, with chromatin in closely packed, varicose strands; prenuclear endoplasmic inclusions: numerous small iron-haematoxylin staining granules, as in *T. quasilli;* postnuclear endoplasmic inclusions: wood, spherules as in *T. sphaerica, T. chattoni,* etc., chondriosomes as in *T. chattoni,* etc.

Host.—Kalotermes (Kalotermes) emersoni Light. Mexico.

Trichonympha saepiculae sp. nov.

Dimensions of body: length 72–149μ, averaging 112μ, width 29–66μ, averaging 48μ; dimensions of rostrum: length in center 11–13μ, diameter at base of cap 6–8μ, diameter at base of collar 11–13μ; ratio of flagellated to non-flagellated region: 0.37:1.00 to 0.75:1.00, averaging 0.55:1.00; flagella as in *T. chattoni;* ectoplasm of flagella-bearing region as in *T. quasilli;* parabasal apparatus of about 40 cords forming a single-layered cylindrical case around the nucleus, and extending for a short distance posterior to it, often split more or less into groups of cords; nucleus at about a third the distance back from the anterior end, 13 (10–17)μ in diameter, with chromatin in irregular, coiled, varicose strands and small nucleolus; prenuclear endoplasmic inclusions: abundance of small, massed granules, enclosed by parabasals, extending back to within a short distance of the nucleus; postnuclear endoplasmic inclusions: spherules as in *T. sphaerica,* small iron-haematoxylin staining granules, wood, etc.

Type host.—Kalotermes (Rugitermes) kirbyi Snyder. Panama.
Additional host.—K. (Rugitermes) panamae Snyder. Panama.

SUMMARY

1. The writer has undertaken to review all systematic and morphological work on the genus *Trichonympha*, to redescribe all species, to describe six new species, and to survey the distribution of the flagellates of the genus in 111 species of termites. Systematic status is given to thirteen species.

2. Only one species of *Trichonympha* has been found in each of most of the infected termites investigated. Exceptions occur in *Reticulitermes* and *Termopsis*. *R. lucifugus* is reported to have two species, but this is not very clearly established. Three species, *T. campanula*, *T. sphaerica*, and the new species *T. collaris*, are present in *Termopsis angusticollis* and *Termopsis nevadensis*. In *Termopsis laticeps*, however, only *T. campanula* has been found.

3. Six entozoic microorganisms are described from the species of *Trichonympha* in *Termopsis*, and four additional ones from other flagellates of the genus. One of these occurs in all individuals of *T. campanula* and *T. collaris*. Another which is found occasionally in the same species is especially interesting in that it is actively motile within the endoplasm.

4. A general account of the morphology of *Trichonympha* as interpreted by the writer is given, beginning on page 425.

5. The detailed morphological observations are summarized in the diagnoses beginning on page 441. A key to the known species, intended chiefly as a summary of the most important distinctions, is given on page 439. The most detailed original morphological studies are those on the three species of *Trichonympha* in *Termopsis*, on *Trichonympha magna*, and on *Trichonympha agilis*.

6. *Trichonympha* has a wide but uneven distribution in termites of the families Kalotermitidae and Rhinotermitidae. It has been found in twenty-seven of seventy-eight Kalotermitidae and in all species of the genus *Reticulitermes*, but not in other Rhinotermitidae.

7. The evidence derived from the comparative morphology and distribution of *Trichonympha* in termites suggests that the genus has undergone but little modification during phylogenetic development of the Isoptera.

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EXPLANATION OF PLATES

The figures were drawn with the aid of a camera lucida except where stated otherwise. Abbreviations for methods of preparation: A., vapor of glacial acetic acid; AC., alum carmine; AF., acid fuchsia; BS., Biebrich scarlet; Ch., Champy's fluid; D., Delafield's haematoxylin; Er., erythrosin; Fl., Flemming's fluid without acetic acid; H., Heidenhain's haematoxylin; K., Kull method after Champy; Os., osmic vapor; R., Regaud's haematoxylin; S., Schaudinn's fluid; Z., Zirkle's copper bichromate fixative.

PLATE 20

Fig. 1. *Trichonympha collaris* sp. nov. from *Termopsis angusticollis*. ✕ 660. Semidiagrammatic. On the left side the surface layers are omitted in order to show the underlying structures. The following structures are represented:

Cap, at anterior end of *rostrum*.

Collar, the ectoplasmic region of the rostrum, limited posteriorly by the *rostral fissure* which extends inward to the *rostral tube*.

Surface ridges, rounded ridges about half as numerous on the rostrum as on the anterior portion of the body.

Flagella, in three zones of different lengths.

Roots of flagella, extending through the ectoplasm of the flagella-bearing zone.

Basal bodies of flagella, rodlets forming a layer at the innermost region of the ectoplasm, fused in the rostral tube. The portion anterior to the nucleus shows the arrangement of these rodlets in rows longitudinally and diagonally.

Blepharoplast, a hemispherical granule at the anterior end of the rostral tube.

Parabasal bodies, typically in groups of three or more, most touching nuclear membrane.

Anterior endoplasmic granules.

Bacteria, in the cytoplasm in the vicinity of the nucleus.

Wood fragments, in posterior endoplasm.

Chondriosomes, abundant in postnuclear endoplasm.
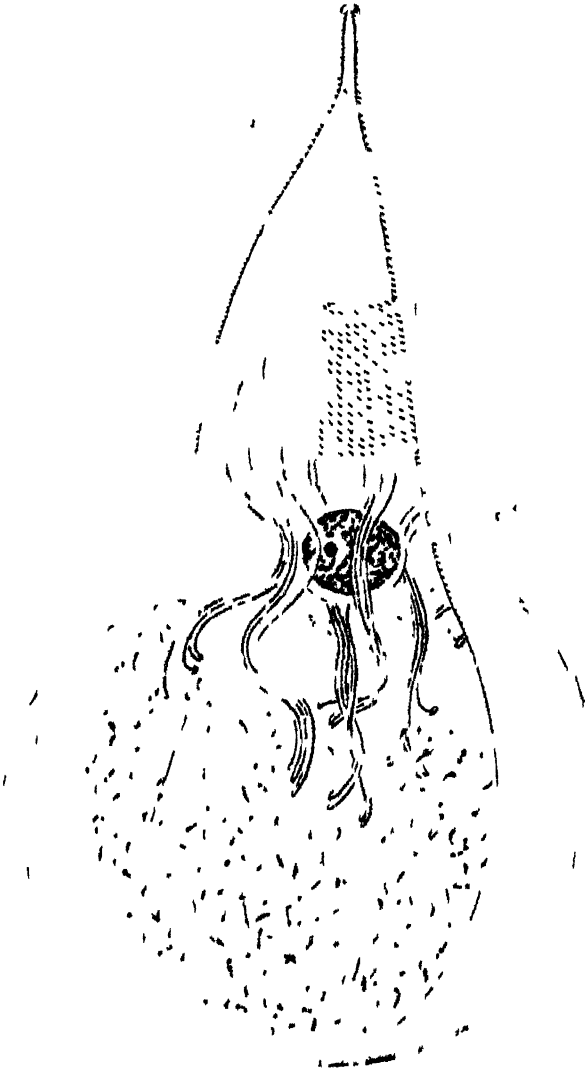
PLATE 21

Figs. 2–5. *Trichonympha campanula* Kofoid and Swezy.

Fig. 2. From *Termopsis laticeps*. Optical section of anterior end, showing cap, rostral tube, blepharoplast, collar, roots of flagella (those of rostrum really extend to the rostral tube). Note the three layers of ectoplasm in rostrum and body, and the dense ectoplasm at the anterior end of the body behind the rostral fissure. S. D. × 880.

Figs. 3–5. From *Termopsis angusticollis*

Fig. 3. Abnormal individual, showing collar separated from the part behind it, ridges on rostrum and body, and granules which are sometimes present in the clear middle layer of ectoplasm. Diagrammatic.

Fig. 4. Optical cross section, showing ectoplasmic layers, form of ridges, origin and roots of flagella, plates. The flagella emerge in the grooves between the ridges. S. H. × 1830.

Fig. 5. Cross-section of compressed individual, showing plates in the outer layer of the ectoplasm, roots of flagella in middle layer, and basal rodlets. There are 100 ridges. Ch. R. × 1335.

Figs. 6–7. *Trichonympha collaris* sp. nov. from *Termopsis angusticollis*.

Fig. 6. Anterior end spread out flat, showing peripheral granules. There are 49 ridges on the rostrum. Ch. K. × 970.

Fig. 7. Rostrum with its parts, layers of ectoplasm, peripheral granules in the outer layer. S. H. × 970.

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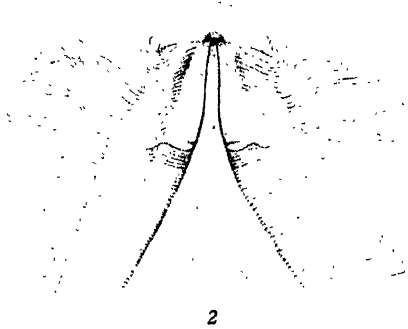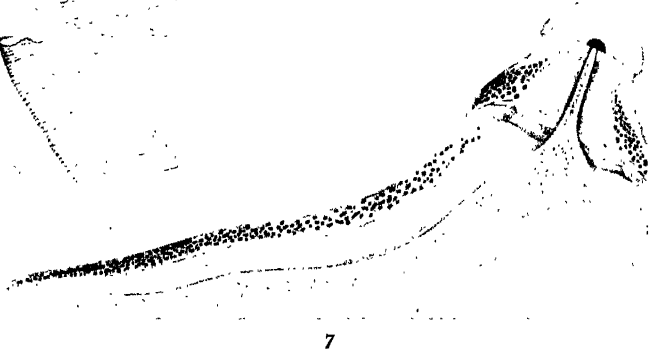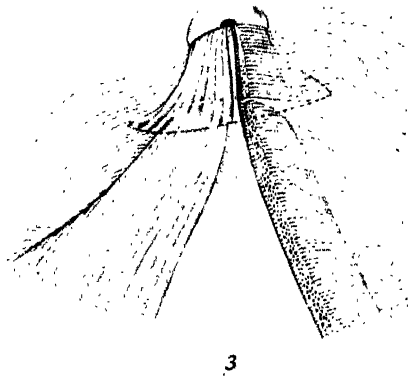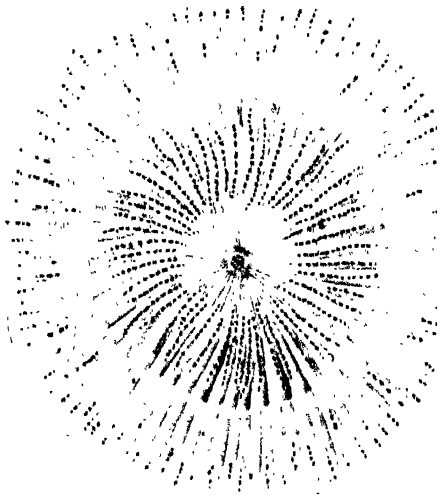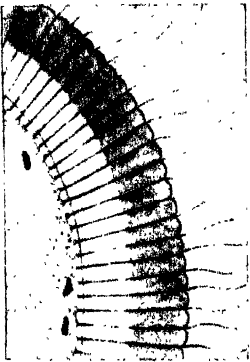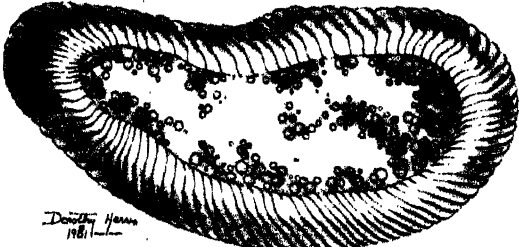
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PLATE 22

Fig. 8. *Trichonympha campanula* from *Termopsis angusticollis*, showing typical arrangement of parabasal cords, anterior endoplasmic granules, and bacteria around and behind nucleus. Ch. H. × 405.

Fig. 9. Same species, showing parabasal cords in vicinity of nucleus, and approximation of some of these to nuclear membrane. Ch. H. × 530.

Fig. 10. Duboscq and Grassé's figures (1927b) showing parabasal structure in *Trichonympha chattoni*. Os. H.

Fig. 11 a-c. *Trichonympha collaris*. a. parabasal thread and clear areas in lightly staining portion. Ch. R. b. section of parabasal, including a group of seven. c. parabasal thread and clear areas. S. H. Diagrams.

Fig. 12. *Trichonympha collaris*. Typical arrangement of parabasal cords, peripheral granules and collar-like appearance of rostrum. Ch. R. × 405.

Fig. 13. *T. campanula*. Surface ridges and anterior endoplasmic granules. The cloudy area near the nucleus represents a mass of bacteria. Z. H. × 615.
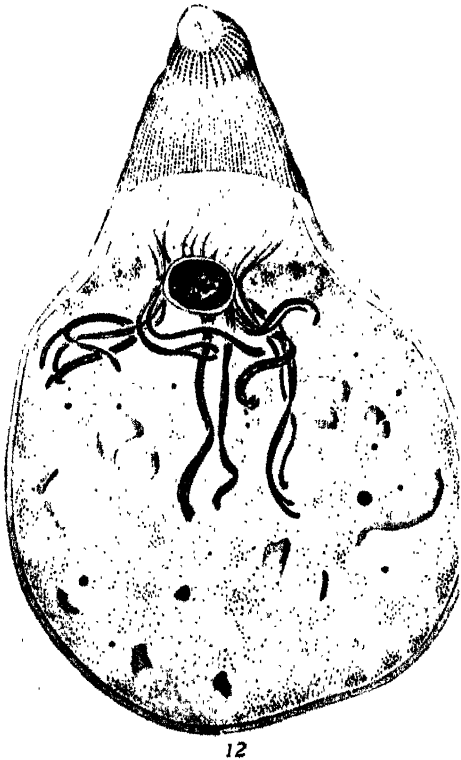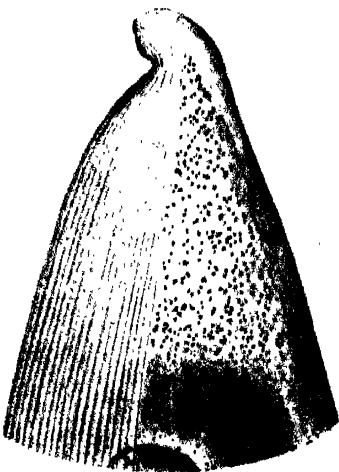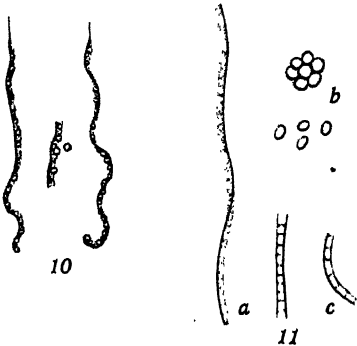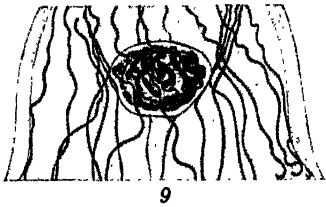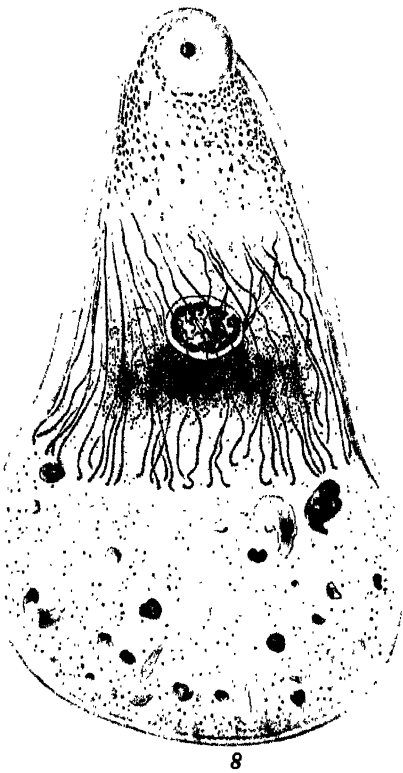
All figures of *Trichonympha campanula*.

Figs. 14–16. From *Termopsis laticeps*.

Fig. 14. Mass of proximo-nuclear bacteria closely gathered around nucleus. Parasites in form of small clustered granules just posterior to this mass. S. D. × 405.

Fig. 15. Mass of proximo-nuclear bacteria anterior to nucleus. S. D. × 405.

Fig. 16. Anterior endoplasmic granules, red; chondriosomes, red; wood in postnuclear endoplasm; parabasal cords. Ch. K. × 405.

Figs. 17–19. From *Termopsis angusticollis*.

Fig. 17. Anterior endoplasmic granules of fig. 13, pl. 22. Z. H. × 1830.

Fig. 18. Portion of individual represented by fig. 13, pl. 22, showing proximo-nuclear bacteria and peripheral position of above granules. Z. H. × 880.

Fig. 19. Enlarged proximo-nuclear bacteria from same specimen. Z. H. × 1830.

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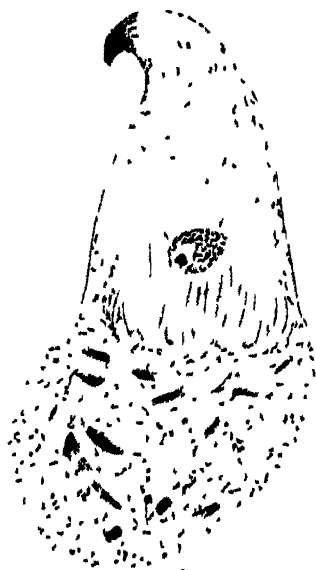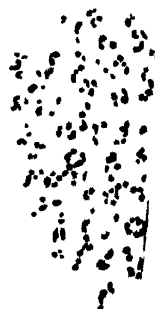
All figures of *Trichonympha campanula*.

Fig. 20. From *Termopsis laticeps*. Parasites in form of small, clustered granules scattered through endoplasm. S. D. × 530.

Figs. 21–24. From *Termopsis angusticollis*, showing peg formed parasite.

Fig. 21. Distribution of parasites. S. H. × 530.

Fig. 22. From same, showing parasites and spherules which are probably digestive residues. S. H. × 1830.

Fig. 23. Peg-formed parasites associated with longer rods, some dividing; the latter probably stages in the life history of former. Ch. K. × 1830.

Fig. 24. Rods dividing into segments shaped like the peg-formed parasites. S.H. × 1830.

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Dorothy G Harris
1931
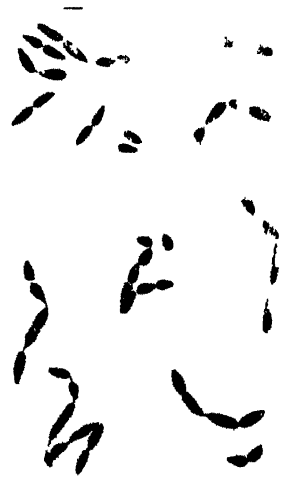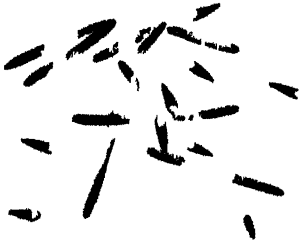
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PLATE 25

Figs. 25–27. *Trichonympha campanula* from *Termopsis laticeps*, showing fusiform parasite. S. D.

Fig. 25. Distribution of parasite. × 530.

Fig. 26. Larger drawing of individual parasite from the same flagellate. × 1830.

Fig. 27. A group of parasites, from one flagellate, showing the range in size down to short, slender filaments. × 1830.

Fig. 28. *Trichonympha collaris.* A portion of the posterior part of the body, showing irregular, rounded, haematoxylin staining masses, probably residues of digestion of other organisms; also wood. S. D. × 530.

Figs. 29–30. *Trichonympha saepiculae* sp. nov. from *Kalotermes (Eugitermes) panamae.*

Fig. 29. Showing typical structure of body and arrangement of parabasals. Fl. D. AF. × 825.

Fig. 30. Optical section of rostrum and anterior part of body, showing blepharoplast, rostral tube, ectoplasmic layers, etc. S. H. × 970.

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28 Dorothy G. Harris
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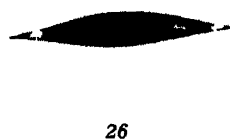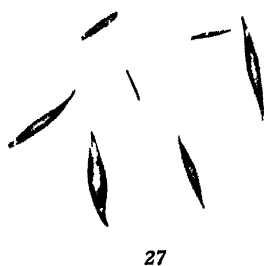
PLATE 26

Fig. 31. *Trichonympha sphaerica* (Kofoid and Swezy) from *Termopsis angusticollis.* The posterior portion of the body is more rounded than is usual. Note the form of the anterior ectoplasm, the length of the flagella, the arrangement of the parabasal cords, the spherules (neutral red staining) in the posterior endoplasm. Composite drawing. × 700.

Figs. 32–34. *Trichonympha sacpiculae* sp. nov. from
Kalotermes (Eugitermes) kirbyi.

Fig. 32. Typical form, hedge-like parabasal apparatus, spherules in posterior endoplasm. Compare fig. 29. S. H. BS. × 700.

Fig. 33. Parasite in nucleus. S. H. BS. × 1104.

Fig. 34. Parabasal apparatus, showing parallel arrangement of cords. S. H. BS. × 947.

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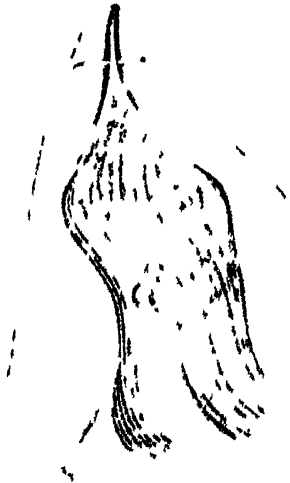
PLATE 27

Figs. 35-37. *Trichonympha sphaerica.*

Fig. 35. Fragments of wood and characteristic neutral red staining spherules in cytoplasm. Some of the latter are granular in interior. A. D. × 530.

Fig. 36. Parabasal apparatus and *Sphaerita*-like parasites. Ch. R. × 530.

Fig. 37. *Sphaerita* from another specimen. Ch. R. × 1830.

Figs. 38-40. *Trichonympha lighti* sp. nov. from *Kalotermes emersoni.*

Fig. 38. Typical form. Note *sphaerica*-like proportions of anterior ectoplasm, parabasal apparatus, small spherules in cytoplasm. S. D. × 825.

Fig. 39. Optical section of rostrum. S. D. × 970.

Fig. 40. Parabasal apparatus. Os. H. × 615.

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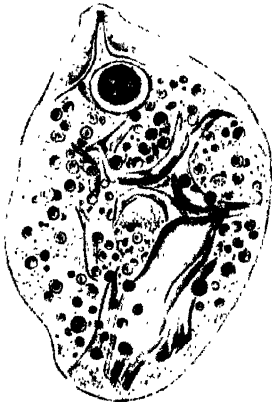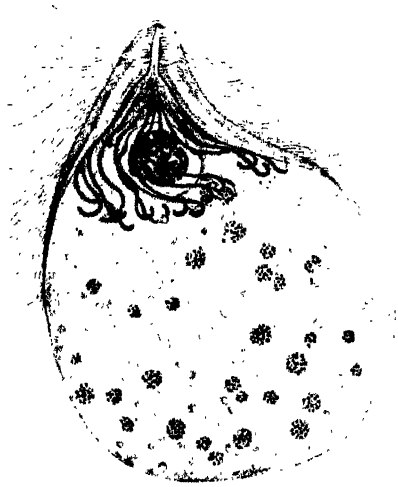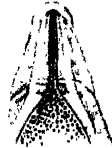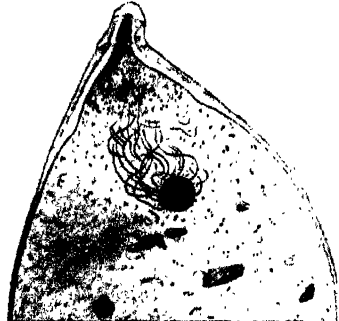
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PLATE 28

Fig. 41   *Trichonympha subquasilli* sp. nov. from *Kalotermes clevelandi*. Anterior end showing structure and characteristic arrangement of parabasal cords. S H. AF.   × 825.

Fig. 42   *Trichonympha quasilli* sp. nov. from *Kalotermes snyderi*. Basket-like arrangement of parabasal cords, and numerous (neutral red staining) spherules in endoplasm   S. H. AF.   × 825.

Figs. 43–48.   *Trichonympha magna* Grassi from *Porotermes grandis*.

Fig 43.   Optical section showing structure.   Note the extensive flagellated zone, the basal granules in transverse rings, the finely granular column in the prenuclear endoplasm, the parabasal apparatus.   Composite drawing.   × 825.

Fig. 44.   Cross-section, showing plates (40), roots of flagella with enlargements, thick ectoplasm, granular column sectioned in center.   S. H.   × 1335.

Fig. 45.   Cross section just behind rostrum, showing a basal granule ring, inner layer of ectoplasm, etc.   S. H.   × 1335.

Fig. 46.   A group of parasites from *T. magna*.   S. H.   × 825.

Fig. 47.   Individual parasites, showing different life history stages.   S. H. × 1830.

Fig. 48.   Cross-section of nucleus and surrounding parabasal cords.   S. H. × 970.
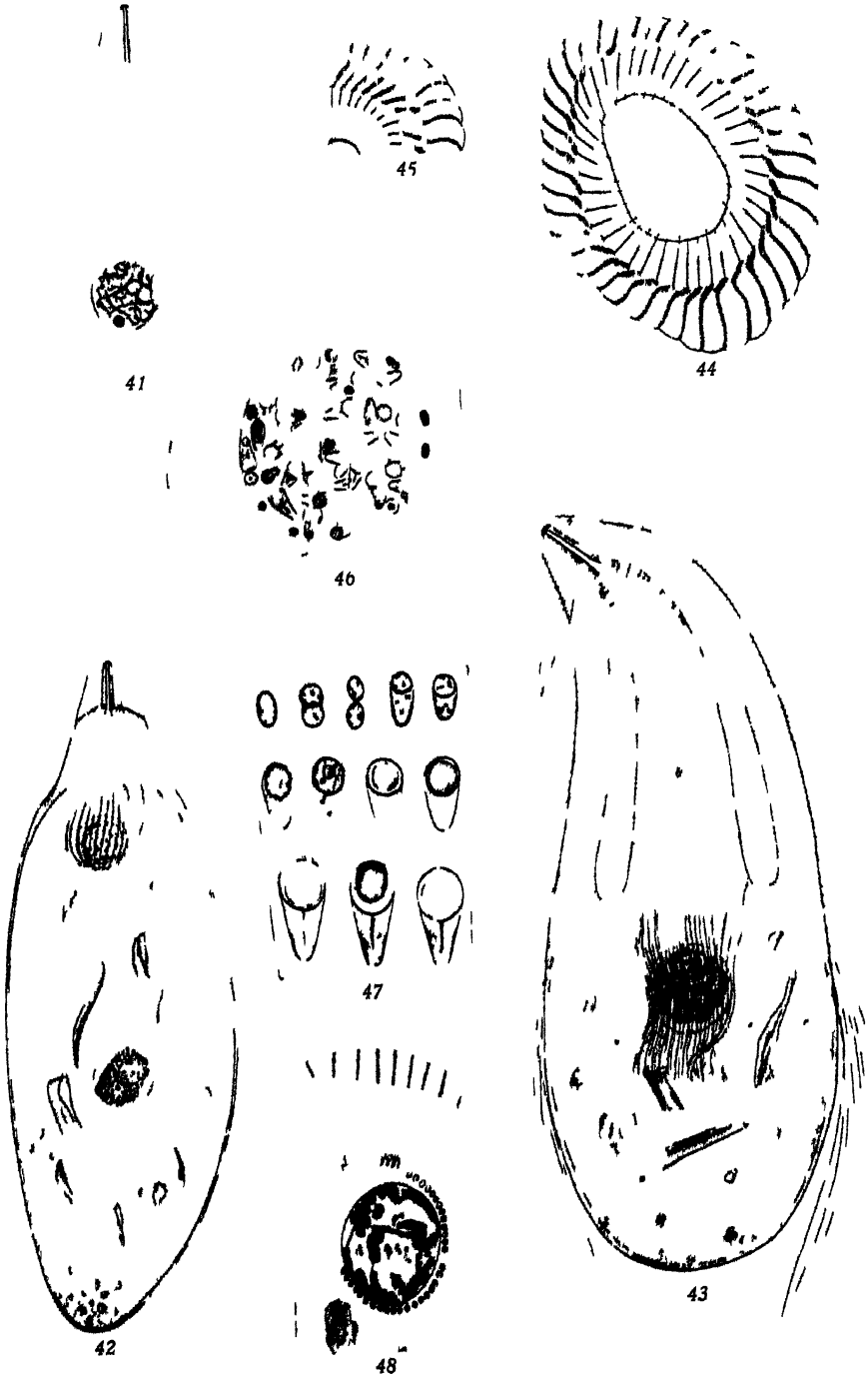
All figures of *Trichonympha agilis* Leidy.

Figs. 49–50. Drawings from living flagellates of
Reticulitermes tibialis. × 825.

Fig. 49. Surface view, showing surface ridges and arrangement of flagella.

Fig. 50. Optical section of same flagellate. The position of the parabasal cords can be seen as clear lines. Anterior to this is a granular ellipsoidal area in the base of which is the nucleus. This stands out clearly in living material.

Figs. 51–53. From *R. flavipes*, showing different arrangements of parabasal cords. × 1335.

Fig. 51. Usual basket-like arrangement. S. H. Er.

Fig. 52. Occasional arrangement, owing to nucleus being pushed posteriorly in body. S. H. Er.

Fig. 53. Unusual arrangement, due to nucleus being further forward than normal. Cap collapsed. Fl. H.

Fig. 54. Rostrum. Fl. H. × 1830.

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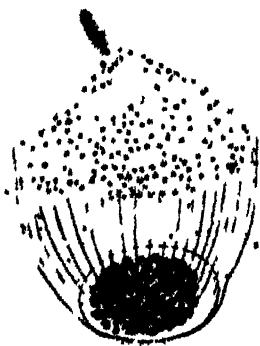
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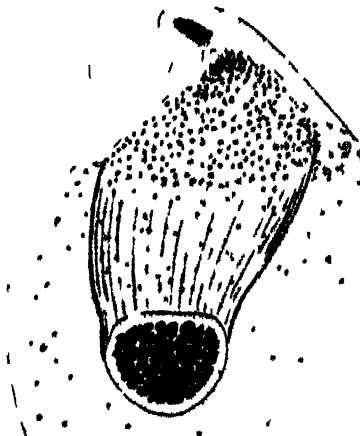
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PLATE 30

Fig. 55. *Trichonympha agilis* from *E. tibialis*, showing numerous endoplasmic granules and bacilliform parasites. Os. H. × 1335.

Figs. 56–69. Nuclei from various species of *Trichonympha*, for comparison. All figures represent the structure of the greater number of nuclei in available specimens, and are believed to be typical for the species and not reorganized prior to mitosis. All × 1830.

Fig. 56.   *T. lighti* from *K. emersoni.*   S. D.

Fig. 57.   *T. quasilli* from *K. snyderi.*   S. H. AF.

Fig. 58.   *T. quasilli* from *K. snyderi.*   S. D.

Fig. 59.   *T. chattoni* from *K. contracticornis.*   S. H. AF.

Fig. 60.   *T. chattoni* from *K. contracticornis.*   S. D. AF.

Fig. 61.   *T. subquasilli* from *K. clevelandi.*   S. H. AF.

Fig. 62.   *T. saepiculae* from *K. panamae.*   S. H.

Fig. 63.   *T. saepiculae* from *K. kirbyi.*   S. D.

Fig. 64.   *T. tabogae* from *K. tabogae.*   S. D.

Fig. 65.   *T. agilis* from *E. flavipes.*   Fl. H.

Fig. 66.   *T. magna* from *P. grandis.*   S. H.

Fig. 67.   *T. sphaerica* from *T. angusticollis.*   S. AC.

Fig. 68.   *T. campanula* from *T. angusticollis.*   S. D.

Fig. 69.   *T. collaris* from *T. angusticollis.*   S. AC.
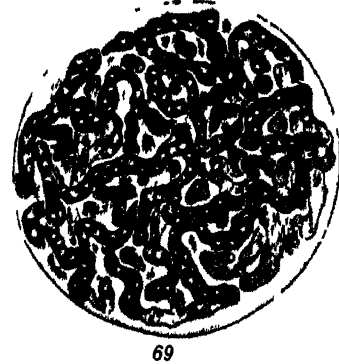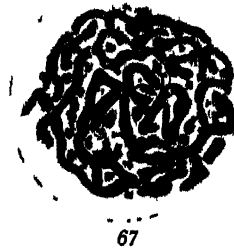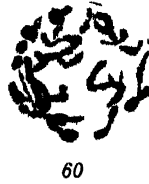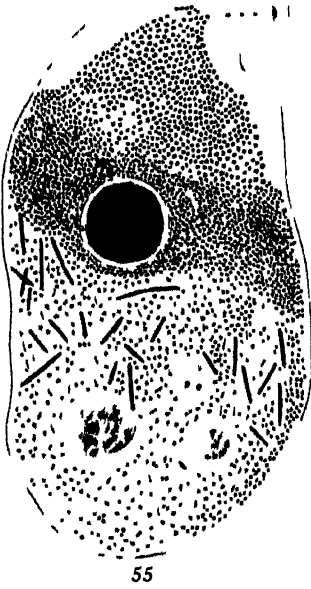
PLATE 31

Microphotographs by J. E. Gullberg.

Fig. 70. Section through the hind-gut of *Termopsis angusticollis*, showing the packed condition of *Trichonympha*. Ch. R. × 34.

Figs. 71–74. *Trichonympha campanula* from *Termopsis laticeps*.

Fig. 71. Individual whose rostrum is represented also by fig. 2, plate 21, showing three layers of ectoplasm, blepharoplast, rostral tube, cap, circular fissure, roots of flagella, etc. S. D. × 584.

Fig. 72. Same, showing nucleus. × 225.

Fig. 73. Individual represented also by fig. 14, plate 23. Mass of proximo-nuclear bacteria closely gathered around nucleus. Parasites in form of small clustered granules just posterior to this mass. S. D. × 223.

Fig. 74. Same, showing parasites more clearly. × 223.

Figs. 75–76. *T. campanula* from *Termopsis angusticollis*.

Fig. 75. Individual represented also by fig. 8, plate 22. Mass of proximo-nuclear bacteria behind nucleus, and parts of the parabasal cords. Ch. H. × 188.

Fig. 76. Mass of proximo-nuclear bacteria anterior to nucleus. Ch. R. × 228.

Fig. 77. *T. campanula* from *Termopsis laticeps*. Alveolar structure in the middle layer of ectoplasm, which appears when the living animal is placed in hypertonic salt solution. This will disappear if the specimen is allowed to recover, but can be preserved in fixed material. S. D. × 254.

Fig. 78. *T. magna* from *Porotermes grandis*. Cross-section represented also by fig. 44, plate 28. Also portion of another individual showing plates and flagellar roots. S. H. × 794.

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ON TOKOPHRYA LEMNARUM STEIN (SUCTORIA) WITH AN ACCOUNT OF ITS BUDDING AND CONJUGATION

BY

ALDEN E. NOBLE

In the title and in the verso folio heading:
For Tokophyra *read* Tokophrya

ON TOKOPHYRA LEMNARUM STEIN (SUCTORIA) WITH AN ACCOUNT OF ITS BUDDING AND CONJUGATION

BY

ALDEN E. NOBLE

ON TOKOPHYRA LEMNARUM STEIN (SUCTORIA) WITH AN ACCOUNT OF ITS BUDDING AND CONJUGATION

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INTRODUCTION

The literature on Suctoria consists largely of scattered and fragmentary observations on the adult morphology of some one hundred and fifty different species of these Protozoa. Most of the observations were so limited by the nature and scarcity of the materials at hand that a survey of their results leaves much to be desired in the way of a clear understanding of the animals. Those records which are more detailed and exact pertain to particular features of morphology or to particular phases of the life-history of organisms which are otherwise poorly known. It is hoped, therefore, that the fortunate discovery of immense numbers of *Tokophrya lemnarum* in all stages of development and activity, may now permit the presentation of a more complete picture of one typical and rather generalized suctorian.

OCCURRENCE

Tokophrya lemnarum was encountered by the writer when in Stockton, California, in February 1930, where it sporadically occurred in laboratory cultures containing water from the Calaveras River. Although a few permanent slides were prepared and some notes on the living animal recorded, no intensive investigation was undertaken until June 1930, when "Lone Tree Pond", located near Berkeley, California, was found to be teeming with the same organisms. This pond proved to be an unfailing source of material for four consecutive months and the study of its yield of *Tokophrya lemnarum* forms the basis of this paper.

HISTORICAL

The adult *Tokophrya lemnarum* was probably first observed by Stein (1854–1859), who, in accordance with the "Acineta Theory" then in vogue, mistook these primitive Suctoria for Acineta stages of at least three different vorticellids. The misinterpretation was corrected by Kent (1880, p. 831) in his Manual of the Infusoria, and the name *Acineta lemnarum* Stein was applied to those "stages" which were reported as occurring on the aquatic plant *Lemna*. The animals were necessarily so incompletely described as to render the inclusion of all of them in a single species a rather questionable procedure. This uncertainty, however, can unfortunately never be dissipated because of the uncertainty inherent in the early observations.

Bütschli (1889, p. 1928) created the genus *Tokophrya* for those Suctoria of the family Acinetidae, which were devoid of cups or tests, more or less pyriform in shape, and with the tentacles confined to the apical end where they were grouped in from one to four bunches. This reclassification automatically withdrew *Acineta lemnarum* Stein to the status of *Tokophrya lemnarum* Stein and it is so listed, without special comment, in Bütschli's monograph (1889).

In an effort to clarify matters, Sand (1898, p. 136) concluded that the designation of a distinct species for *Tokophrya lemnarum* was unwarranted by the meager facts at his disposal and interpreted Stein's figures to be representations of *Tokophrya cyclopum* Claparède and Lachmann (1858–61), with which, indeed, *Tokophrya lemnarum* has several features in common. Accordingly, the name *Tokophrya lemnarum* was reduced to a synonym of *Tokophrya cyclopum*.

Sand's disposal of *Tokophrya lemnarum* could not well be disputed without an actual rediscovery of the discarded species. Fortunately Entz, Sr. (1903) encountered a species of *Tokophrya cyclopum* which more closely approximated Stein's figures of what was later called *Tokophrya lemnarum*. He (1903, p. 108) accordingly reestablished the species *lemnarum* and distinguished it from *Tokophrya cyclopum*, in substance, as follows:

The body of *Tokophrya lemnarum* averages much larger in size than that of *Tokophrya cyclopum* and it is bounded by a thick integument which is frequently wrinkled in marked contrast to the smooth surface of the latter species. The pedicel of *Tokophrya lemnarum*, unlike the relatively short one of *Tokophrya cyclopum*, is much longer than the body; and the macronucleus which is spherical in *Tokophrya cyclopum*, tends to be ovoid or elongated in *Tokophrya lemnarum*.

To Entz, then, we owe the first statement of the distinctive features of *Tokophrya lemnarum,* but the description was incomplete in some particulars. Moreover, it is the belief of the writer that the hitherto accepted description of *Tokophrya lemnarum* contains a major inaccuracy regarding an important diagnostic feature of the species. Attention is therefore called to this error before a new, or rather amplified, description is offered. This inaccuracy, which for three months caused the writer to believe that he was dealing with a new species, is the alleged possession by *Tokophrya lemnarum* of from *three to five micronuclei.*

Now, the micronuclei of these animals are extremely difficult to demonstrate. They are exceedingly minute, and in the resting state they adhere so closely to inconspicuous depressions in the macronucleus that they escape detection in the usual preparations. In the vast majority of whole mounts, even the oil immersion lens fails to reveal any micronuclei whatsoever. Properly prepared sections (see p. 482) of *Tokophrya lemnarum,* however, definitely show that the animal possesses *one and only one micronucleus.*

Neither Collin (1912) nor Penard (1920) picture micronuclei in their figures of *Tokophrya lemnarum,* so it may be assumed that Entz is the authority for their statements that the animal possesses from three to five. Turning to Entz's (1902) publication, we find three figures which do contain three, four, and five "micronuclei" respectively. These "micronuclei," however, are not only more numerous than the present work indicates, but they are larger, of various sizes, and in one figure at least, occupy positions at some distance from the macronucleus. It is also to be noted that Entz mentions no difficulty in staining the "micronuclei".

There are only two possible explanations for these contradictory data. Either there is a confusion of two distinct species or Entz has mistaken certain other structures in the animal's cytoplasm for micronuclei. The latter explanation seems to the writer to be more probable for two important reasons. In the first place, many Suctoria, including *Tokophrya lemnarum,* contain bodies which closely simulate micronuclei. These are the fragments of ingested nuclei within the minute food vacuoles which are characteristic of the group. When very numerous and when found in animals which are surrounded with abundant food their true nature is easily understood, but when found in fixed material of moderately fed animals their number and size frequently render them indistinguishable from ordinary micronuclei.

Many of the writer's prepared slides show specimens of *Tokophrya lemnarum* which conform to the figures of Entz, but in these cases the small, densely stained bodies are indisputably not micronuclei, but food vacuoles containing chromatin.

In the second place, to declare the *Tokophrya*, which forms the subject matter of this paper, to be a species distinct from *Tokophrya lemnarum* might also necessitate the withdrawal of the species discussed by Collin and Penard with which the species under consideration agrees in every other particular. While such a procedure might not be without some justification, the questionable status of the "micronuclei" in Entz's figures does not warrant so drastic an act.

It should be reiterated that, in at least many species of Suctoria, chromatin-containing food vacuoles very closely simulate micronuclei. Failure to differentiate clearly between these two structures has been responsible for similar confusion throughout the literature.

TECHNIQUE

Laboratory cultures for the study of living material were found to be easily maintained in Syracuse watch glasses and in larger culture dishes to which edible Protozoa were added daily and in which excessive accumulation of organic materials was prevented. These cultures survived as a rule for about five weeks without any special difficulties.

Dark field illumination is by far the most satisfactory method by which to study the living animals. The individual tentacles and the contours of the body are much more sharply outlined than is possible with ordinary illumination, and the animals are sufficiently small to permit observation of several internal structures.

Flotation of coverslips occasionally entrapped young individuals; but because of the small size of the larvae (30 microns), their speed of locomotion, and the rapidity of their metamorphosis, it was necessary to devise a technique which would instantly stop the larvae at selected moments of activity or metamorphosis. In order to accomplish this end a physiological inductorium was connected to the material on the slide in such a way that the organisms under observation were compelled to move about between two very fine electrodes. With careful adjustment of the secondary coil, a current could be produced which, at the touch of a key, would instantly paralyze or kill the animals yet not cause immediate disintegration. Although the technique was not uniformly reliable in results, it permitted many rapid

observations of stationary larvae in various stages of development and also made possible the use of high power lenses for such observations.

Materials for permanent mounts were fixed in Schaudinn's, strong Flemming's, Zenker's, and Bouin's fixatives. Whole mounts were stained in Feulgen's fuchsin-sulphurous-acid reagent, borax carmine countered with indulin, and alum carmine. Sections were stained in iron-hematoxylin, phosphotungstic-acid-hematoxylin, Ehrlich's hematoxylin, Feulgen's fuchsin-sulphurous-acid reagent, safranin, and Sharp's modification of Mallory's triple connective tissue stain for Protozoa. Phosphotungstic-acid-hematoxylin after Bouin's fixative was found to be the most suitable for micronuclear studies, while the best general preparations were made from Schaudinn-fixed material stained in iron-hematoxylin. Only cold Schaudinn's can be used successfully, since hot solutions of any kind affect the basal cement in some way, causing the animals to become detached.

Feulgen's stain, while brilliantly coloring the macronucleus, was disappointing in its failure to disclose micronuclei. As has been described, the feeding mechanism of *Tokophrya lemnarum* made this failure inevitable, inasmuch as food vacuoles containing pure nuclear material took the stain as intensely as did the micronuclei of the suctorian. In fact, owing to the finely subdivided and scattered nature of the chromatin in the swollen, active micronuclei, most nuclei were more difficult to see with Feulgen's stain than with many other stains which colored more than the chromatin. It was found impossible adequately to study micronuclear behavior in any but sectioned material stained to a certain optimum degree with phosphotungstic-acid-hematoxylin. Incidentally this technical experience may be taken as a warning against over-reliance on the Feulgen nuclear test.

MORPHOLOGY

THE ADULT

Tokophrya lemnarum is extremely variable in size and shape. What may be designated as a typical *Tokophrya lemnarum* is an animal shaped somewhat like a conventionalized heart whose apex is fastened to a delicate stalk which is about three times the length of the body (pl. 32, fig. 1; fig. A, 1). A flattening of the body causes the animals to present different faces. When viewed along the edge, the impression of a heart disappears and the animal would more correctly be described as a club (fig. A, 3). Broad grooves down the flattened

face give rise to yet another contour which is presented when the
animal is viewed from its superior end. This view suggests the outline
of a dumbbell (fig. A, 2). Tentacles radiate from the shoulders of the
heart, forming two distinct bunches at the apical end.

The average measurements, as obtained from two hundred typical
animals, are: length of body 53 (18–104)μ; width of body 38
(12–82)μ; thickness of body 24 (7–54)μ; length of stalk 154 (131–
167)μ; diameter of stalk 3.48 (3.2–3.7)μ; length of tentacles 58
(34–75)μ; diameter of tentacles 0.4 (0.3–0.5)μ.
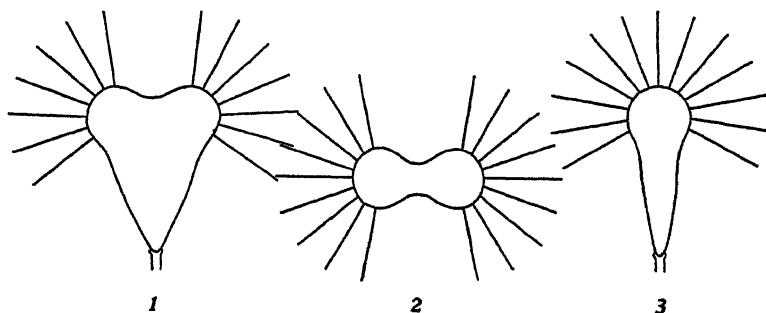
Fig. A.   Views of *Tokophyra lemnarum.* See text.

General morphology—

While a little more than 50 per cent of the animals encountered
were of the above described typical structure, *Tokophrya lemnarum*
is so variable in size and shape that almost half of them bore little
specific resemblance to the typical form. Indeed, it was the writer's
first impression that several distinct species were under observation.
Most of the variations seemed to fluctuate about three main types
which represent as many distinct phases in the life-history of the
organism.

There were, in the first place, the plump, rounded, animals which
had recently fed and were consequently distended in a smooth, sym-
metrical fashion (pl. 32, fig. 2). These forms averaged larger in size
than the typical ones, the actual size depending upon the amount of
food ingested. It was not uncommon to find well fed individuals from
five to ten times the size of unfed animals. Tentacles of these larger
forms, instead of being confined to two distinct regions, uniformly
covered the entire superior ends of the bodies.

There were, secondly, the asymmetrical forms in the peculiar
stages preparatory to conjugation (pl. 33, fig. 1). One or more body

protuberances, later described as conjugative processes, served to modify the contours of the bodies in all conceivable manners.

Finally, there were a large number of animals which had recently given birth to ciliated embryos. These exhibited an irregularly collapsed integument enclosing a rather shapeless mass of protoplasm (pl. 33, fig. 2). Such forms were usually appreciably smaller than the average in size.

In addition to these three main modifications which were constantly present in all but starved cultures, many other peculiarities of form were noted. The body was frequently seen to be immensely elongated with an accompanying decrease in diameter and a close bunching of the tentacles into a single group at the apex (pl. 32, fig. 3). The two bunches of tentacles were, in other cases, subdivided into four groups precisely as in the normal condition in *Tokophrya quadripartita* (pl. 32, fig. 5). The two, in rare cases four, shoulders bearing the tentacles were occasionally seen to be prolonged into definite arms, somewhat simulating the tentacled arms of the Dendrocometidae.

While these particular peculiarities may be wholly anomalous and never again be encountered, the fact worthy of note is that variously modified and bizarre *Tokophrya lemnarum* may be expected in collections of this species, and that descriptions of suctorian species based on observations of a small number of individuals may contain serious errors.

The *integument* of *Tokophrya lemnarum* is thick and of uniform consistency throughout; and while it is sufficiently elastic to permit enormous engorgement during feeding, it will not shrink beyond a rather definite point. Consequently small and underfed animals, and animals which have just discharged their embryos present wrinkled surfaces, which give the illusion of being tests like those of *Acineta*.

The *pedicel* is composed of at least three layers each of different material (pl. 36, fig. 1). There is an extremely thin outer pellicle of transparent substance, an intermediate sheath composed of from sixteen to twenty-four delicate rods, and an inner core of homogeneous substance which, with some difficulty, can be demonstrated by certain stains. The outer pellicular layer does not appear to be continuous with the integument of the body, although its extreme thinness does not permit a positive denial of such a possibility. In any case, its staining reactions indicate a difference in constitution from that of the integument. The layer of rods gives the pedicel its striated appear-

ance both in life and, more markedly, in stained material. The lower end of the stalk is imbedded in a homogeneous substance in the shape of a disk which serves to cement the pedicel to the substrate and the upper end is slightly enlarged in the form of a funnel which receives the basal curvature of the body. Although the writer was unable to discover the precise method of attachment of the body to the stalk, his observations incline him toward the view of Collin (1912, p. 109) as stated in regard to other Suctoria, that the rods terminate distally in tiny cups into which some elements of the animal integument, perhaps minute prolongations, are inserted. The stalk, like those of all other known Suctoria, is non-contractile and possesses no powers of movement of its own. If mechanically bent, it will regain its rigidly straight position immediately upon release of the bending agency.

The *tentacles* of *Tokophrya lemnarum* number from ten to twenty-four in each of the two bunches. Although very flexible and highly contractile under suitable stimulation, the writer has never seen them move save when stimulated by the presence of prey or by some mechanical disturbance. For the most part they remain extended in a manner which is remarkably rigid for threads of such delicate structure. There is a distinct enlargement of the tentacle at its distal end where it widens into a minute funnel with thickened wall; but the nature of the enlargement does not seem to merit the term "capitate", which has been frequently used in designating similar enlargements of other suctorian tentacles. They are cupped rather than capitate.

Optical sections of the living tentacle clearly demonstrate its tubular nature; there is an outer wall continuous with the limiting membrane of the animal body and an inner wall lining the apparently hollow tube (pl. 35, fig. 4). The inner wall is prolonged for a short distance into the cytoplasm of the body where it abruptly terminates.

A fully contracted tentacle is somewhat thicker than an extended one, and it is regularly wrinkled and lined in such a way as to give the appearance of being spirally wound upon itself (pl. 35, fig. 4). The spiral lines at times appear to be at least partly composed of minute granules but their small size renders this point problematical.

The *cytoplasm* in life is finely and uniformly granular, and shows a tendency of the granules to concentrate in a peculiar pattern in the center of the cell (pl. 32, fig. 1). Fixed and stained cytoplasm has, at times, the appearance of a fine network throughout which granules of different sizes are scattered; and at other times it has merely the living granular appearance much intensified.

A single *contractile vacuole* pulsates directly beneath one of the tentacled protuberances or, in the more rounded forms, toward the apical end of the animal but to one side of the median line (pl. 32, figs. 1, 2). The average size of the vacuole just prior to contraction is 8.5 microns.

The *macronucleus* is slightly ellipsoidal or spherical in shape, averaging 10.56 (8.62–12.2) microns in its longest axis. It is usually located in the center or slightly toward the apical end of the body (pl. 32, fig. 1) ; but, as will be described later, it is readily pushed into other positions, and during such pressure as that encountered during body contractions, even its shape may be greatly distorted. Both living and stained material show the resting macronucleus to be composed of dense granules of fairly uniform size and shape compressed within a clearly visible nuclear membrane (pl. 35, fig. 7; pl. 38, fig. 4).

The single *micronucleus* is invisible in life. In suitably prepared material, as indicated above, it is seen as a minute granule (3μ) lying close to the macronucleus and usually in a small depression in the macronuclear membrane where it escapes detection in all but sectioned material. A clear halo, limited on its outer circumference by a definite membrane, can usually be seen to surround the granule (pl. 36, fig. 2).

Food vacuoles measure from three to five microns in diameter. They occur in numbers of from none at all in unfed animals, to several hundred in animals so engorged with food as to preclude the possibility of accurate counting. They are of two types: those which stain but slightly darker than the cytoplasm and others, less numerous, which stain as deeply as do the nuclei. The cause of so marked a difference in staining reactions will be discussed under the section on feeding.

THE LARVA

The larva of *Tokophrya lemnarum* is spheroidal to elongate in shape with the basal end, i.e., the end which progresses forward during locomotion, having a slightly larger diameter than the end which is destined to become the apical portion of the adult (pl. 33, fig. 6). Four bands of cilia encircle the body in a region slightly below the secondary axis, and a tuft of about six cilia arises from a point near the future apical end, thus giving the larva a definite bilateral symmetry. The basal end, as revealed by dark field illumination, is

crowded with closely packed granules and is occasionally seen to bear a small rounded prominence at its tip. There is, however, no evidence of a ring or rudimentary stalk. The apical end, i.e., the end which is posterior in locomotion, usually terminates in a shallow pit as if in anticipation of the adult depression between the groups of tentacles. The larva does not possess a mouth and it has never been seen to bear tentacles while free-swimming.

The cytoplasm, like that of the adult, is finely granular and frequently contains a few food vacuoles derived from the parent. The macronucleus is likewise comparable in composition to the adult macronucleus, but it is more spherical and somewhat smaller in size. The single micronucleus, invisible in the living animal, occupies a position closely adherent to the macronuclear membrane and consists, in stained material, of a dense granule surrounded by a clear halo which is externally bounded by a definite nuclear membrane. Unlike the adult, the larva possesses two alternately pulsating contractile vacuoles (pl. 33, fig. 3). These are of equal size and more or less constantly located on opposite sides of the macronucleus and toward the future apical end of the body.

The average measurements of the larva are as follows: length of body 37 (31–44)μ; thickness of body 30 (21–36)μ; diameter of nucleus 9 (8–12)μ; diameter of contractile vacuoles 8 (7–11)μ; length of cilia about 12μ.

ECOLOGY

Lone Tree Pond, the source of the *Tokophrya* studied, is a stagnant but permanent pool about one hundred feet in diameter (during the time collections were made), which is shaded by trees over the larger portion of its area. The duckweed, *Lemna gibba,* grew thickly at the surface of the pond and the smartweed, *Polygonum* sp., grew abundantly near the shoreline. The water lily, *Alisma plantago,* and the matted rootlets of the willow, *Salix babylonica,* also fringed the pond, although much less abundantly. *Spirogyra* sp. occurred rather sparsely at various points in the water.

It is a remarkable fact that *Tokophrya lemnarum,* which was originally named after the *Lemna* to which it was attached, occurred on the roots of all the plants named above excepting *Lemna gibba.* It also occurred occasionally on débris such as bits of wood and dead, submerged leaves; but by far the most abundant collections were made from *Alisma plantago,* whose roots never failed to yield an abundant

supply. As a rule, those plants in the more shaded areas, especially those areas hemmed in by matted roots or other vegetation, proved to be the best collecting grounds.

BEHAVIOR

Movements of Body

A phenomenon which, to the writer's knowledge, has not yet been reported as occurring among the Suctoria, is the power of movement which the body of *Tokophrya lemnarum* manifests. While for the most part, the adult animal remains rigidly stationary over long periods of observation, individuals are occasionally seen to bend or twist slowly upon their pedicels, in such manner as completely to alter their configurations. Flexures may occur in any region of the integument (pl. 35, fig. 5) causing the animal to bend upon itself at any possible angle, but the most common pivot of movements is that more or less narrow region of the body adjoining the stalk. Flexures at this point permit the animal to incline itself in any direction and at the same time maintain elsewhere its previous contour (pl. 35, fig. 6).

It is not within the province of this paper to suggest a mechanism for suctorian movements; but it may not be amiss to recall that the non-contractile vorticellids possess powers of bodily movements which are comparable to the movements above described; perhaps movement on the part of certain Suctoria is a primitive characteristic and a further evidence of affinities with the Vorticellidae.

Feeding Activities

The food of *Tokophrya lemnarum* was seen to consist chiefly of *Euplotes patella, Paramecium caudatum, Paramecium multimicronucleata, Oxytricha* sp., and various detached vorticellids. Attempts, frequently noted, to devour attached vorticellids always failed because of the powerfully contractile stalks of the latter, and in the case of the non-contractile vorticellids, the bodies grew well out of reach of the suctorian tentacles.

If, as has been reported for other Suctoria, *Tokophrya lemnarum* possesses any powers of paralysis over its prey, the writer has seen no evidence of this in many observations on the feeding process. The tentacles seize instantly any acceptable ciliate which happens to come in contact with them and remain firmly attached to it while the prey

struggles, rarely successfully, to free itself. The strength of the tentacles is truly astonishing when they are seen in action. An animal several times the size of its captor can be held by two or three delicate-appearing threads (pl. 34, fig. 7) against the most vicious lashings of heavy cirri and repeated jerks for liberty. Curiously enough, tricho-cysts have never been seen to discharge from *Paramecium* during such struggles. Relatively few tentacles are involved in a given capture, and of these, only from three to ten function as actual suckers. Although probably all of them can serve as mouths, the majority which take part in feeding, merely serve to hold the prey in position. There is an interval of from a few seconds to a full minute from the time a tentacle is attached to the time food passes through it. What transpires during this interval of time is unknown but it is obvious that the pellicle of the prey must in some manner be dissolved or ruptured before suction can begin. If, however, there is an actual penetration into the cytoplasm, it is for too short a distance to be evident under an oil immersion lens.

The tentacles which are to serve as cytostomes gradually enlarge (pl. 35, fig. 4) to a diameter twice or more than twice that of the non-sucking tentacles. An examination under high powers of these enlarged tentacles reveals a rapid stream of granules flowing from the cytoplasm of the prey to the internal limits of the tentacles where, in the same manner as in any holozoic infusorian, food vacuoles are formed (pl. 35, fig. 4). The fully formed vacuoles leave the ends of the tentacles in rapid succession and move about the body in an apparently haphazard manner.

The nucleus of the prey usually remains intact until the cytoplasm has all but been ingested when it, in turn, is drawn in by the tentacles and sucked into food vacuoles which, however, differ from the pre-ceding ones in that they contain only nuclear material. These are the food vacuoles which, after being stained, are easily confused with micronuclei.

There seems to be no limit to the tokophryan appetite. Once an animal is seized, regardless of its size and regardless of the time of previous feeding, suction continues so long as there is a body left from which to suck. Consequently a small *Tokophrya* may engorge itself until, within the space of ten minutes or less, it is many times its original size. The entire process takes from two to twenty minutes, depending upon the size of the prey, *Euplotes patella* being completely ingested by an average-sized *Tokophrya* in about fifteen minutes.

In the meantime the attacked protozoan has been undergoing an interesting process of destruction. The body grows smaller and rounder while the integument, instead of becoming noticably wrinkled, evinces a remarkable constancy and elasticity. Locomotor organelles continue to beat vigorously until the body has been reduced to a small fraction of its normal size, although coordination of their activities soon ceases. The cirri of *Euplotes patella,* by way of example, beat in an apparently autonomous manner long after the body has been reduced to an unrecognizable residue and even its nucleus has been devoured. In fact, it would appear that the cirri continue to beat until they, individually, begin to disintegrate, regardless of the state of disintegration of the rest of the body.

It has been reported by Penard (1920, p. 137) that *Tokophrya lemnarum* appears to rid itself of the remnants of its repasts by a peculiar ejective performance on the part of the tentacles. Particles of débris appeared to him to be pushed aside by adjacent tentacles until the immediate vicinity was swept clear of all residue. During the writer's observations, however, *Tokophrya lemnarum* gave no evidence of such behavior. Much of the débris was incidentally removed as the tentacles became disentangled from each other and extended in the characteristic radiating fashion, and this process of readjustment frequently gave rise to an illusion of a housecleaning behavior. Once the tentacles regained their normal extended positions, however, any remnants which were left about their bases continued to lie there undisturbed.

ENCYSTMENT

Two types of suctorian cysts have been described. More transitory and partly protective coats may be formed about the body or, frequently in the same species, a harder and more or less angular and patterned coat may be formed for longer and for seasonal periods of adversity. Only the former type of cyst has been seen for *Tokophrya lemnarum* (pl. 35, figs. 7–10). On the sixth of July a watch-glass culture was found to contain numerous encysted animals and animals in various stages of encystment. The entire process was not actually observed for an individual animal, but the various stages present left little doubt as to the chief steps in the process. A gelatinous secretion occurs about the body, gradually increasing in thickness until a thick transparent coat envelops the entire animal. The apical portion of the body appears to be the last to participate in the secretory process;

consequently many animals in the early stages of encystment possess cup-like additions to their bodies not unlike the "loges" of various other species of the Suctoria (pl. 35, fig. 7). Indeed the similarity is so marked as to suggest the possibility that such cups or "loges" of the higher Suctoria originated through arrested development of cyst walls—a sort of permanent semi-encystment that results in greater protection.

The tentacles are not withdrawn, but become inert and matted over the apical surface (pl. 35, fig. 8) where they are eventually engulfed by the growing cyst wall. Whether they persist within the wall or whether they disintegrate as the gelatinous substance comes in contact with them, is as yet impossible to say; although, as soon as completely formed, the cyst wall appears transparent and homogeneous throughout.

The young cyst is plump, smooth, and colorless. About 10 per cent of the cysts appeared slightly darker and somewhat wrinkled at the surfaces, possibly an indication of greater age or maturity. The body within the cyst is spherical, smaller in diameter than its active relatives, and the cytoplasmic granules are so densely packed as to render the nuclear outline indistinct.

Excystment was not observed and no cysts were seen detached from their stalks. It is not as yet known whether or not the cysts normally become detached and thus aid in the dispersal of the species, but the fact that *Tokophrya lemnarum* has appeared at the College of the Pacific in laboratory cultures made with clear water from the Calaveras River to which dried organic materials were added, is strongly suggestive of an affirmative answer.

REPRODUCTION

FORMATION OF THE EMBRYO

The first indication of budding in a living *Tokophrya lemnarum* is an elongation of the macronucleus; but, as shown in stained preparations (pl. 36, fig. 3), this elongation is preceded by a micronuclear division and is accompanied by the appearance of a narrow slit-like cavity in the apical region of the body. This demarkation of the cytoplasm progresses downward in the form of a hemisphere (pl. 36, fig. 4) upon which four bands of cilia early make their appearance in line with the main axis of the body. The elongated macronucleus, in the meantime, grows larger at the upper end by a migration of

granules to this region from the center of the nuclear mass thus presenting, roughly, the figure of an hourglass (pl. 36, fig. 4). By the time the demarkation of cytoplasm is completed, i.e., by the time a small ball has been virtually cut out of the parent cytoplasm, the macronucleus is severed into two unequal parts, the smaller part rounding into the macronucleus of the bud and the larger part regaining its normal shape in the parent cytoplasm. The respective micronuclei come to lie against the macronuclei and the formation of the bud is completed. Shortly before the bud is severed from the parent cytoplasm, the two contractile vacuoles described above make their appearance.

While this process is commonly referred to as "internal budding", it must be noted that the expression can be correctly applied only to the behavior of the macronucleus. The embryo itself arises through a sort of engulfment of a portion of the parent cytoplasm including such food vacuoles and other elements of the cell as happen to be present. The double-walled method of embryo formation results in an embryo lying within a definite brood pouch. The writer has been unable to determine whether this brood pouch persists throughout life in an extremely contracted condition save for reproductive periods, or whether it arises *de novo* at each birth. Sections, however, fail to reveal any evidence of a permanent brood chamber and its method of formation demonstrates that, permanent or not, there is no connection between the chamber and the parent pellicle until the formation of the embryo is completed. This fact precludes the possibility of an invaginative process from the parent integument.

DISCHARGE OF THE EMBRYO

Immediately after the embryo is cut off from the parent cytoplasm, it begins to rotate within the brood chamber at right angles to the main axis of the parent body and its cilia are clearly seen vibrating in the narrow space between the embryo and the wall of the brood chamber. The embryo has no means of leaving the parent on its own initiative; the parent expels it by a process remarkably suggestive of mammalian labor. After the embryo has rotated freely for from two to thirty minutes, the parent body enters upon a series of increasingly strong contractions. The pressure against the brood chamber obliterates the space between it and the embryo and causes all movement to cease, eventually even preventing pulsation of the contractile vacuoles. With each successive contraction the parental body becomes more

wrinkled and distorted in shape and it is frequently seen to bend and twist upon its pedicel in a manner that can only be described as writhing. The nucleus becomes distorted, is thrust to various parts of the body, and the food vacuoles and cytoplasmic granules are so violently agitated as to present a picture of complete disorganization.

Gradually the embryo is pushed to a position midway between the groups of tentacles and against the integument of one side of the body in such a way as to cause a pronounced protuberance. Whether or not a permanent pore for the egress of the embryo exists, one always begins to appear at precisely the same point on each reproducing animal, a point midway between the tentacles and at the tip of the protuberance. Sooner or later the birth pore widens and the embryo itself begins to protrude, basal end foremost. When half-emerged and when the cilia first make their appearance on the outside of the parent (pl. 33, fig. 4), there is a sudden release of the pressure against the parental contractions and the embryo is literally shot from the pore so forcibly that the eye can follow it only with the low power objective. Like a gaping wound, the partly collapsed brood chamber remains evident in the parent for several minutes (pl. 35, fig. 1). Gradually, however, and usually within the course of half an hour, all evidence of reproductive activities disappears and the parent becomes indistinguishable from its non-reproducing neighbors.

LARVAL BEHAVIOR

The only purpose of larval freedom is to insure suitable attachment for the perpetuation of a sessile race. A free-swimming larva of *Tokophrya lemnarum* utilizes its few moments of liberty only to affix itself to some substrate as quickly as possible, a goal which is usually attained in less than a minute and within a few millimeters of its parent. Basal end foremost, the larva spins on its axis as it darts first in one direction and then in another without a pause between turns. Each time it comes in contact with solid material, such as root hairs, the coverslip, or particles of debris, it stops its forward locomotion, presses its basal end against the object, and modifies its rotation to a peculiar wobbling as though the animal were slightly off center at its point of contact. That this behavior is the method of larval attachment is further evidenced by the fact that a sticky secretion appears at the base which, if the larva is unsuccessful in its efforts, trails after it in the form of viscous strands, collecting microscopic particles of debris which greatly impede locomotion. Many futile

attempts at attachment are usually made in rapid succession, before the animal finally succeeds in becoming fixed (pl. 33, fig. 6). The fate of a larva which is unable to find a point of fixation is unknown; but whereas metamorphosis always begins at the moment of attachment, those larvae which were experimentally prevented from attachment for over an hour showed no signs of metamorphosis at the time they finally died or became lost.

METAMORPHOSIS

Perhaps the most astonishing feature of larval development is the speed of metamorphosis. It ordinarily takes less than an hour from the time of attachment to the time the animal attains full adult status, and within six minutes the pedicel grows from an undifferentiated basal region of the larva to a structure three times the length of the body—a rate of growth which permits actual observation through the microscope. The cilia cease to beat within two minutes and tentacles make their appearance all over the surface of the body just before the pedicel reaches its maximum length (pl. 33, fig. 7). The tentacles reach their normal length in about fifteen minutes.

The rate of metabolism during the rapid metamorphosis of the young *Tokophrya* is doubtless greatly increased, and the possession of two contractile vacuoles is probably associated with the need for increased excretory activity. At any rate, after the tentacles and the pedicel have attained their maximum lengths, one of the two contractile vacuoles begins to pulsate less frequently and gradually ceases functioning altogether. No evidence of a second contractile vacuole has been seen in a fully metamorphosed animal.

For from fifteen minutes to three-quarters of an hour the young *Tokophrya* bears tentacles scattered regularly over the entire surface of the body and the pedicel appears to be inserted for a distance in the basal end (pl. 33, fig. 7). Eventually, however, the tentacles are confined to the apical end by a process of evagination of the basal portion of the animal which is devoid of tentacles and are separated into two groups by a symmetrical double evagination at the superior end.

The following table summarizes a typical history of larval development as it was observed July 28, 1930:

9:06 A.M.—Evidence of internal budding first noted
10:57 A.M.—Detachment from parent cytoplasm noted; independent rotation
       took place

11:04 A.M.—Parental contractions began
11:07 A.M.—Embryo rotations ceased
11:08 A.M.—Contractile vacuoles obliterated
11:25 A.M.—Embryo protruded from birth pore
11:25¼ A.M.—Embryo escaped
11:26 A.M.—Larva attached to root
11:27¾ A.M.—Cilia stopped beating
11:30 A.M.—Stalk measured three times length of body
11:33 A.M.—Tentacles appeared
12:01 P.M.—One contractile vacuole disappeared.
12:16 P.M.—Tentacles were longer than body
12:30 P.M.—Metamorphosis was completed

CONJUGATION

HISTORICAL

The superficial features of contact and union between two members of the same species of Suctoria were noted and variously interpreted by several of the early protozoologists (notably Bütschli 1889, p. 1914), but the first indications of a nuclear reorganization as an essential feature of the phenomenon were independently discovered by Schneider (1886) and Plate (1886, p. 194). No micronuclei were observed by either of these authors; and endeavoring to account for the reappearance of a macronucleus after an unmistakable fragmentation and absorption of the original one, they postulated a creative rôle for the cytoplasm itself. While this erroneous hypothesis did not meet with wide acceptance, it served to focus attention on conjugation among the Suctoria and gave rise to an interesting controversy over the significance of the phenomenon.

Sand (1898) became the chief exponent of the idea that conjugation among the Suctoria "consiste essentiellement en une plastogamie" without "le moindre mélange nucléaire" (p. 154). If there is any macronuclear fragmentation, he declared, it is "pour se baigner dans le cytoplasme rénové et se reconstituer ensuite" (p. 154). The existence of micronuclei anywhere in the group was completely denied.

Maupas (1889), on the other hand, championed the idea that an act of conjugation is in itself a reliable indication of the presence of micronuclei and that conjugation among the Suctoria follows the same general course of events as that observed among the ciliates. In support of his contention he described in part an act of conjugation in *Podophrya fixa* without, however, giving any figures.

Both these points of view were supported largely by indirect evidence for the very good reason that micronuclei were and still are extremely difficult to demonstrate in at least the vast majority of Suctoria.

To Koeppen (1880) we owe our first knowledge of a micronuclear spindle occurring in the Suctoria. In his description of *Acineta papillifera*, he presented two figures of what were unmistakably micronuclei in a pair of conjugating individuals; and he stated that, although he was unable to trace the sequence of events, his observations led him to believe that a new macronucleus was formed by the metamorphosis of a product of micronuclear division.

That conjugation among the Suctoria differs in no essential respect from that among the ciliates was definitely proved by Hickson and Wadsworth (1902) in their work on *Dendrocometes paradoxus*. These authors have demonstrated the regular occurrence of two prezygotic and two postzygotic micronuclear divisions during the conjugation of *Dendrocometes paradoxus;* and although they express uncertainty in regard to the later stages of the process and in regard to the precise method of formation of the new macronucleus, their account of conjugation remains the most detailed and complete to date. ˙

The next contribution to our knowledge of suctorian conjugation is contained in the work of Martin (1909) on *Acineta papillifera.* The author ascertained with certainty the existence of two prezygotic and two postzygotic divisions of the micronucleus. As alternative hypotheses concerning the method of macronuclear reformation, he suggests (1) that two of the four postzygotic nuclei, after undergoing partial metamorphosis, fuse to form the new macronucleus while one of the remaining two becomes the permanent micronucleus, and the other disintegrates; or (2) that two of the postzygotic nuclei disintegrate while the remaining two form the permanent micronucleus and macronucleus respectively. Most of Martin's figures of micronuclei, like my own, resemble faintly stained vacuoles.

Finally Collin (1912), in his masterly monograph on the Acinetaria, has pictured micronuclei and micronuclear spindles in several different species of Suctoria, together with various stages of macronuclear disintegration and reformation. He has not, however, outlined the entire process for any one species.

* Since this paper went to press, additional observations on conjugation in *Dendrocometes* have been reported by Bruno, Pestel, Beiträge zur Morphologie und Biologie des *Dendrocometes paradoxus* Stein. Archiv für Protistenkunde, 75(3): 403–471, 55 figs. in text.

OBSERVATIONS ON LIVING CONJUGANTS

Rarely were less than 2 per cent of the *Tokophrya lemnarum* collected by the writer in the act of conjugation, and occasionally collections netted as much as 10 per cent of conjugating animals. Whatever may be the prompting stimulus or the physiological state which predisposes the animals to conjugation, the phenomenon occurs at all ages and during any condition of nourishment. Small organisms devoid of food vacuoles conjugate with each other or with large well fed animals as freely as do the animals engorged with food. Although the adult *Tokophrya lemnarum* ordinarily ignores the presence of larvae of the species as indifferently as it does the presence of inedible ciliates, a larva was once seen to fuse with an adult *Tokophrya* (pl. 34, fig. 6) and to remain fused for nine hours, after which time the pair was unfortunately lost. While micronuclear behavior could not be seen, the act was undoubtedly one of conjugation. On the other hand, that some peculiar physiological state or environmental agency is prerequisite for the phenomenon is demonstrated by the absence of conjugation among animals in close proximity concurrent with the presence of conjugants which succeed in reaching each other in spite of wide separations.

As previously stated, the preconjugant comes in contact with another animal of the species by bending toward it or by sending out heavy pseudopodial processes. While it is possible that the presence of another member of the species somehow constitutes a directive stimulus for the conjugative act, many animals have been seen with conjugative processes extending in directions other than that of the nearest individual which may be manifesting the same futile behavior. Indeed this phenomenon is so common that if it is not the result of a random reaching for a mate, mutual attraction between specific, often widely separated pairs would have to be postulated.

When two *Tokophrya* or their conjugative processes reach each other (pl. 34, fig. 2) fusion promptly takes place and the animals are drawn closer together through a retraction of the processes. As a result the pedicels become bent, or in rare cases, one is actually pulled loose from its base. Any portion of the surface of the body, except the tentacled areas, can take part in the fusion phenomenon but a membrane always persists between the two animals and there is never an admixture of cytoplasms. In cases where a well fed animal conjugates with a small one devoid of food vacuoles, the food

vacuoles remain on their side of the partition throughout the entire process of conjugation.

Early stages of conjugation do not appear to alter the feeding or other normal behavior of the participants, but as the conjugants gradually separate the tentacles begin to wilt and to cease functioning (pl. 34, fig. 4). With release from the last point of contact, the animals snap back to their original positions by virtue of the elasticity of their pedicels and they soon lose all trace of tentacles. The macronucleus eventually becomes so reduced in size or so fragmented that, even with low power objectives, living exconjugants can usually be recognized by their peculiarly opaque appearance.

It was found difficult to time accurately the entire process of conjugation; but such records as it was possible to make show that it is not completed in less than twenty-four hours and that forty-eight hours is probably sufficient time for its completion in Berkeley at summer temperatures of about 60° to 70° F. At any rate, after a lapse of forty-eight hours, no evidence of the phenomenon could be found in animals which had been isolated during the early stages of conjugation.

NUCLEAR PHENOMENA

The active micronuclei of *Tokophrya lemnarum* are rounded or slightly elongated bodies which average twice the size of ordinary food vacuoles but the range varies from slightly smaller to four times the size. Consequently the difficulty of recognizing them as micronuclei is enormously increased. They must be distinguished from the two kinds of food vacuoles, on the one hand, and the larger contractile vacuoles and vacuoles seen only in preparations after fixation, on the other. In late stages, which frequently harbor fragments of disintegrating macronuclei, the difficulty is further augmented.

The reason for this difficulty, which exists regardless of the fixative and stain used, is not clear; but the present work suggests that the discrete elements of the micronucleus, which are relatively large in most ciliates are, in *Tokophrya lemnarum* at least, actually below the limits of visibility under the oil immersion lens. If these elements are all contained within the minute granule of the resting micronucleus which is itself seen with difficulty, a dispersion of the elements over an area several times the size of the resting nucleus necessarily carries them beyond optical detection. It may well be, therefore, that the only possible means of observing micronuclear behavior in an animal such as this, is the utilization of whatever slight differences

in fixation and staining reactions may exist between micronucleoplasm and the surrounding cytoplasm.

Since the accompanying figures, like most other published figures of suctorian micronuclei, differ so greatly from those we are accustomed to see in conjugating ciliates, a list of six criteria for the identification of such nuclei is here offered.

1. They must be found only in conjugating and budding individuals; and, in the latter case, they should not number more than two.

2. They should range in size from slightly smaller to more than twice the size of the food vacuoles present and in color from that of a clear vacuole to a slightly darker shade than that of the cytoplasmic food vacuoles. It should be remembered in this connection that these sizes are relative and can not be ascertained directly with a micrometer rule. All structures in a large animal are larger than those in a smaller one; hence the food vacuoles in a large animal may be quite as large as active micronuclei in the smaller one.

3. They should occasionally show minute dark staining particles (chromosomes?) within a definite membrane and, quite as frequently, faint striations which give the body the appearance of a spindle. Food vacuoles, on the contrary, should be homogeneous in content regardless of degree of digestion.

4. Careful study of the history of both types of food vacuoles should at no point show structures simulating these larger bodies.

5. A study of the animals containing the suspected bodies should reveal a conformance to Maupas' generalized outline of the conjugative process; or, at least, not do violence to it.

6. Comparisons with published figures of suctorian conjugation should further convince one of the validity of his interpretations.

Regardless of the time it takes for the protruded processes to reach each other, no evidence of nuclear reorganization is manifested until actual contact between these processes of the conjugants has occurred. For descriptive purposes, the nuclear changes occurring during conjugation in *Tokophrya lemnarum* may be arbitrarily divided into twelve successive stages.

Stage 1. *Micronuclear swelling.* The micronucleus of each conjugant leaves its position closely adherent to the macronuclear membrane and undergoes a gradual enlargement. Incipient swellings of the micronucleus can be readily seen because of a still more or less concentrated condition of the chromatin within it (pl. 37, fig. 1), but upon completion of the swelling process the micronucleus measures

from five to eight microns in diameter and appears as a faintly stained vacuole (pl. 37, fig. 2). No definite nuclear spindles have been seen, the nearest approach to this condition, which is so common in ciliates, being an occasional oval-shaped micronucleus showing very faint longitudinal striations (pl. 37, fig. 3).

Stage 2. *First prezygotic division.* The single micronucleus in each conjugant divides into two similarly swollen micronuclei (pl. 37, fig. 2) without, so far as the writer has been able to ascertain, giving rise to the elongated dumb-bell structures characteristic of dividing micronuclei in ciliates. The swollen nucleus appears to become simply constricted into two parts in a manner analogous to binary fission. At about the same time, the macronucleus exhibits a more compact concentration of granules within its center, thus revealing a clear area between the nuclear membrane and the compact central mass (pl. 37, fig. 3; pl. 38, fig. 6).

Stage 3. *Second prezygotic division.* In the same manner in which the first division occurred, a second division gives rise to four prezygotic nuclei (pl. 37, fig. 3). Those nuclei, however, which play no further part in the conjugative process very quickly degenerate and, consequently, individuals simultaneously containing all four micronuclei are rarely seen.

Stage 4. *Formation of germ nuclei.* One of the four prezygotic nuclei divides a third time (pl. 37, fig. 3), giving rise to gametes which, though somewhat smaller than the other nuclei show no sexual dimorphism. The remaining three nuclei quickly disintegrate. The changes progress in the macronucleus to a point at which it is somewhat spindle-shaped and is traversed by coarse striations (pl. 38, fig. 7).

Stage 5. *Union of germ nuclei.* Actual fusion of the gametes has not been seen. One figure (pl. 37, fig. 3), however, shows an unmistakable penetration of a gamete from one conjugant into the cytoplasm of another which has clearly formed its own germ nuclei. While fusion undoubtedly occurs immediately after the exchange of gametes, the act of fusion may be of such short duration that it practically escapes fixation. Hickson and Wadsworth (1902) and Martin (1909) report that gametic fusion in *Dendrocometes paradoxus* and in *Acineta papillifera* probably takes place in the resting stage, but in *Tokophrya lemnarum* true resting micronuclei have not been seen in any phase of the micronuclear process. The elongation of the macronucleus continues (pl. 38, fig. 8) until it is two or three times as long as broad

and the striations become finer, fainter, more numerous, and closer together.

Stage 6. *First postzygotic division.* Save that all unmistakable postzygotic divisions occur only in conjugants which have separated, the first postzygotic division presents a picture like that of the first prezygotic division. In many of the preparations studied, however, it has seemed to the writer that products of postzygotic divisions average slightly darker in coloration than those that occur prior to fusion of the germ nuclei, and that the postzygotic nuclei also show discrete particles within them more frequently than do prezygotic nuclei. These somewhat doubtful appearances may result from the presence of a diploid number of chromosomes which would reveal their presence more readily than would be possible before the gametes fused. Does, then, reduction occur at the first or second prezygotic division? Since it is the writer's firm conviction that it will never be possible actually to count the chromosomes of this species, this is a question which he deems beyond the possibility of a direct answer in *Tokophrya lemnarum* with the evidence available.

Stage 7. *Second postzygotic division.* This stage, one of the most common in the writer's materials, gives rise to four typically swollen micronuclei (pl. 37, fig. 5). The macronucleus frequently shows the first signs of actual disintegration in this stage by a dissolution of a portion of the macronuclear membrane and an attendant flow of granules into the cytoplasm (pl. 38, fig. 9).

Stage 8. *Third postzygotic division.* The discovery of exconjugants with five, six, and seven micronuclei early made it evident that at least three micronuclear divisions took place after the formation of the zygote. A prolonged search for all of the eight micronuclei, however, has resulted in but one figure (pl. 37, fig. 6) to corroborate the conclusion. Absence of other figures is probably again owing to the short duration of those micronuclei which play no further rôle in the conjugative process. It is rather surprising to find three postzygotic divisions in a process which results in but two products, a single macronucleus and a single micronucleus.

Stage 9. *Final micronuclear division.* One of the eight products of zygotic division probably undergoes a final division destined to result in the permanent micro- and macronucleus. This statement is made with some hesitancy because the single figure upon which it is based (pl. 37, fig. 6) has not been verified by others. This figure, however, clearly shows nine nuclei, two of which were in a state of

chromatin condensation quite different from the remaining seven which were presumably on their way to extinction. The figure, furthermore, fits in excellently with subsequent events.

Stage 10. *Early metamorphosis of macronucleus and micronuclei.* One of the final products of division quickly becomes smaller by virtue of a condensation of its chromatin into a small compact granule characteristic of the resting micronucleus (pl. 37, fig. 7). The other product of division, destined to become the macronucleus, is early recognizable by the presence of two or three faintly staining granules within it (pl. 37, fig. 7; pl. 38, fig. 2). These granules are positively characteristic of the metamorphosing macronucleus from the time it begins to metamorphose until it is indistinguishable from the resting nucleus of the species.

Stage 11. *Later metamorphosis of the macronucleus.* The new macronucleus gradually increases in size. As it does so the granules within gradually increase in number; but the staining properties of the nucleus undergo an even more gradual change, causing it to remain as a faintly outlined sphere (pl. 37, fig. 8) until the old macronucleus is almost absorbed. During growth, minute darker stained specks occasionally appear in the nucleus (pl. 38, fig. 3) only to disappear in a homogeneous background before metamorphosis is complete. The writer is unable to account for this phenomenon.

Stage 12. *Disintegration of old macronucleus.* During the metamorphosis of the new macronucleus and often continuing after its completion, the old macronucleus passes through a series of changes which, although not in uniform pace with the micronuclear changes, presents a constant sequence of events. Unlike the events in micronuclear behavior, these macronuclear events are rendered optically vivid. The observable history of these changes is essentially a history of alignments and transformations of granules. What appear to be networks and lines of fibers under lower powers of magnification are, under the oil immersion lens, resolved into alignments of granules in an otherwise homogeneous nucleoplasm. At this time the greatly elongated old macronucleus becomes so closely curved about the growing macronucleus that the new nucleus appears to be budding out of the old (pl. 37, fig. 8). This illusory configuration is probably of no conjugative significance but is brought about by the mechanical pressure of the surrounding cytoplasm which causes the elongated structure to conform to the large sphere which is usurping its former position in the center of the body.

Sooner or later the macronuclear membrane ruptures and the enclosed granules pour into the surrounding cytoplasm (pl. 38, fig. 9). Instead of scattering indiscriminately throughout the body, however, they tend to coalesce near their point of emergence in a few compact lumps which gradually assume the appearance of irregularly vacuolated masses (pl. 38, fig. 10). These masses do not fragment indefinitely but grow gradually smaller, lose their staining power, and become absorbed in a manner analogous to the digestion of material in food vacuoles. Remnants of the old macronucleus are rarely seen in animals whose functional macronuclei have attained the typical vegetative appearance.

The slow increase of stainability of the metamorphosing macronucleus is so intimately correlated with the disintegration and loss of staining power of the old macronucleus as to suggest a necessary relationship between the two processes. If, as Hickson and Wadsworth (1902, p. 351) state, "there is no inconsistency in the view that after the disappearance of the old meganucleus (in *Dendrocometes paradoxus*) its nucleoplasm is still living in a modified form diffused through the cytoplasm", it is quite possible that the developing macronucleus gradually incorporates the nucleoplasm of the old macronucleus into its own substance, thus regaining the staining properties possessed by the functional macronuclei.

Conjugation in *Tokophrya lemnarum*, unlike that which occurs among ciliates, is not directly associated with reproduction. This point of dissimilarity seems to be associated with the peculiar budding powers of suctorian nuclei; because, while conjugation in ciliates usually, if not always, extends over a period of time involving more than one generation of animals, a daughter *Tokophrya* seems to require a macronucleus which has arisen as a bud from a functional parental nucleus. In its essential features, however, conjugation in *Tokophrya lemnarum* conforms to the generalized scheme of infusorian conjugation as formulated by Maupas (1889); and the close parallel manifest in these most vital phases of protozoan life-cycles is confirmatory evidence of the relationship between ciliates and the Suctoria.

SUMMARY

Tokophrya lemnarum is found to be extremely variable in shape and size. The body most commonly ranges from spherical to pyriform in shape with a marked tendency to assume the contour of a heart at its broadest surface. The average measurements of the body are 53 (18–104) × 38 (12–82) × 24 (7–54) microns. The integument is thick and frequently wrinkled. The striated stalk, most diagnostic of the external features, is about three times the length of the body. The tentacles, slightly longer than the body, are typically concentrated in two bunches at the apical end; they are cupped and not "capitate". There are no tests and no "loges".

The macronucleus, spherical to ellipsoidal in shape, is definitely granular in life as well as in fixed preparations. Contrary to previous erroneous reports, there is but one micronucleus, which is so small and so closely adherent to the macronuclear membrane that it usually escapes detection in all but sectioned material. Food vacuoles containing chromatin have probably in the past been mistaken for micronuclei. There is a single contractile vacuole located under one of the groups of tentacles.

The *larva* is spheroidal to elongate in shape, measuring 37 (31–44) × 30 (21–36) microns, and it is banded by four rows of cilia near the center of the body. A tuft of cilia is also borne near the future apical end. There are two contractile vacuoles, and as in the adult, one macronucleus and one micronucleus.

The *ecology* of *Tokophrya lemnarum* reveals a marked preference for *Alisma plantigo* as sites of attachment and a surprising failure to utilize the abundant *Lemna gibba* after which the species was named. The animals always occurred in close association with various species of vorticellids.

Certain types of *behavior* of the living animals are reported; their capacity for movements upon the pedicels being recorded for the first time. Feeding activities and the formation of the two types of food vacuoles are described in detail. Paralysis of the prey does not occur.

Encystment is described. Liberation of the cysts from their stalks has not been observed.

The *embryo* arises by a spherical extension of a slit-like cavity which appears near the apical region of the body. This extension cuts out a portion of cytoplasm embracing a macronucleus, which has been budded from the parental nucleus, and a product of micronuclear division. There is no invagination of a brood chamber from the integument.

The embryo is discharged through a temporary birth pore by a succession of contractions of the parent body. Larval life, however, lasts only long enough to secure suitable attachment, usually less than a minute; and metamorphosis to the adult state is completed in less than an hour.

Conjugation as observed in *Tokophrya lemnarum* is described. The animals achieve mutual contact by means of pseudopodial extensions called "conjugative processes". Between the time just prior to separation of the conjugants and the complete nuclear reorganization, the tentacles wilt and disappear.

Since active micronuclei are easily confused with various cytoplasmic vacuoles, six criteria for the recognition of such nuclei are offered. Two prezygotic and three postzygotic divisions of the nucleus are demonstrated. One of the eight products of postzygotic division divides a fourth and final time to give rise to the new macronucleus and micronucleus. Metamorphosis of the new and disintegration of the old macronucleus are shown to exhibit a constant sequence of transformations. Unlike conjugation among ciliates, the macronuclei of immediately following generations never arise directly as a feature of the conjugative act. Buds derive their macronuclei only by virtue of the budding powers peculiar to the suctorian nucleus. The close parallel of the essential features of conjugation, however, is confirmatory evidence of the relationship between ciliates of the Suctoria.

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EXPLANATION OF PLATES

All figures were drawn with the aid of a camera lucida except figures 4 and 7–10, in plate 35, which are composite and schematic drawings. Plates 32–35 represent living animals; plates 32–34 show their appearances with dark field illumination. All figures in plates 36–38 represent stained whole mounts, except pl. 36, figs. 2, 5, and 6; pl. 37, fig. 1; pl. 38, fig. 9; which represent stained sections. The following abbreviations for methods of preparation are used: B., Bouin's fluid; F., Feulgen's nuclear stain; Fl., Flemming's fluid; H., Heidenhain's haematoxylin; S., Schaudinn's fluid; Saf., safranin; P., phosphotungstic-acid-haematoxylin.

PLATE 32

Fig. 1. Typical *Tokophrya lemnarum* showing nucleus, contractile vacuole, and aggregation of cytoplasm in peculiar central pattern. × 357.

Fig. 2. Animal which has recently fed, showing numerous food vacuoles and a grouping of tentacles in single bunch at the apical end. × 357.

Fig. 3. Elongated type of the species with single bunch of tentacles. × 357.

Fig. 4. Spherical type. × 357.

Fig. 5. *Tokophrya lemnarum* with four bunches of tentacles. × 357.
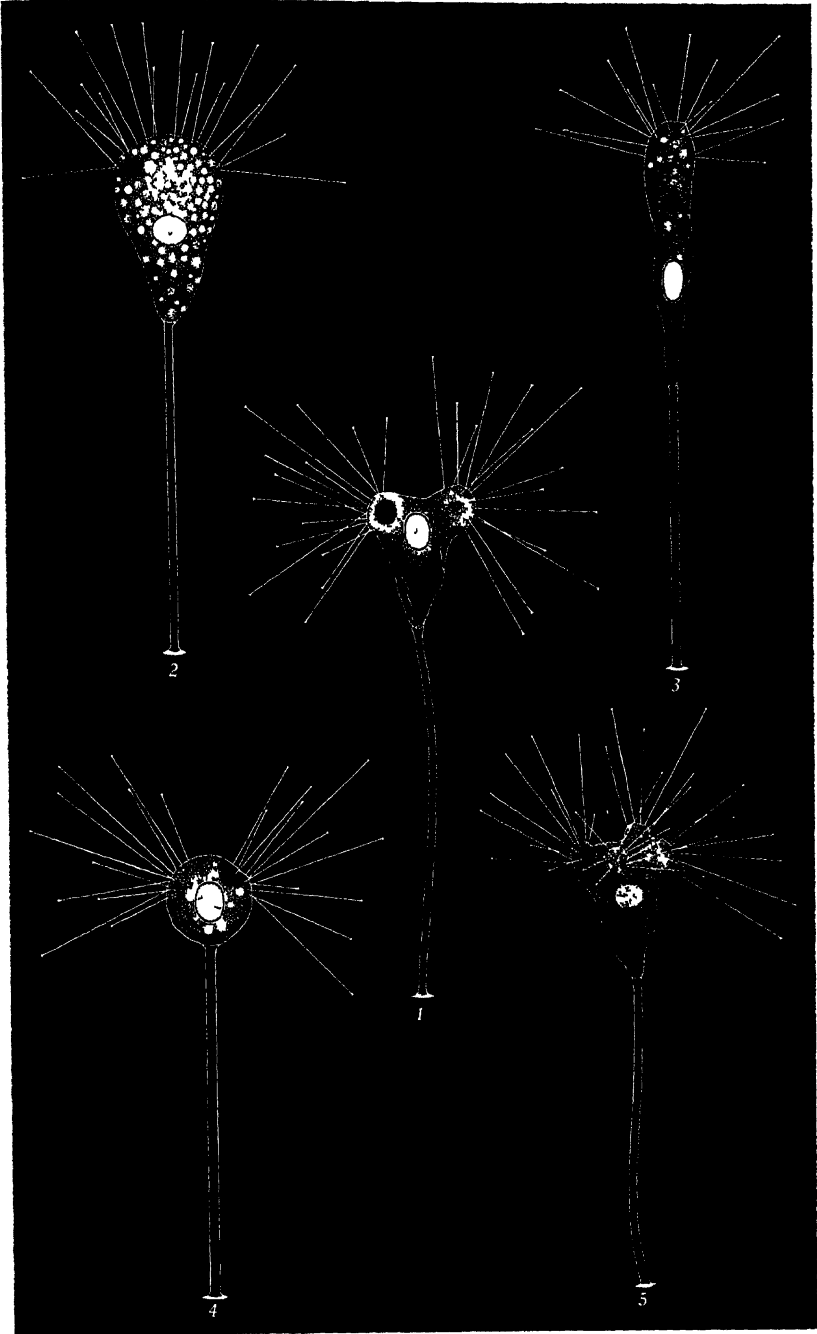
Fig. 1.   Asymmetrical animal with two conjugative processes.   × 357.

Fig. 2.   Asymmetrical animal which has recently given birth to larva.   × 357.

Fig. 3.   Animal containing embryo within brood pouch.   × 357.

Fig. 4.   Embryo partly discharged from birth pore.   Note contractions of parent body.   × 357.

Fig. 5.   *Tokophrya lemnarum* larva with four bands of cilia about body and a tuft of cilia at apical end.   × 357.

Fig. 6.   Larva immediately after attachment to substrate.   × 357.

Fig. 7.   Early metamorphosis of larva, showing increase in length of pedicel and appearance of tentacles.   × 357.

Fig. 8.   Late stage of larval metamorphosis.   The tentacles have not yet been separated into two groups.   × 357.
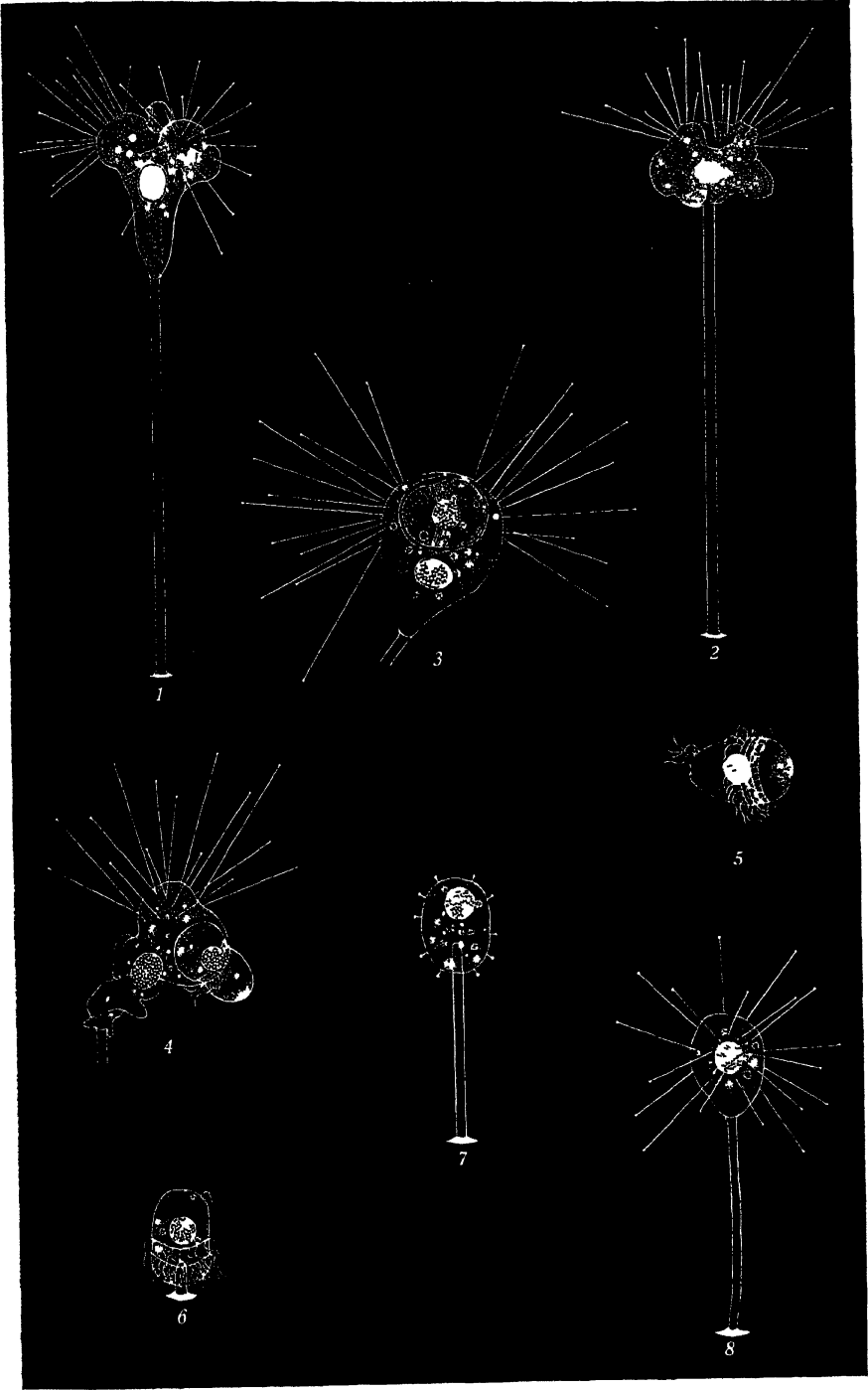
PLATE 34

Fig 1   Preconjugants bent toward each other.   × 357.

Fig 2   Fusion of conjugative processes.   × 357.

Fig. 3   Pair of completely fused conjugants.   × 357.

Fig. 4   Partial separation of conjugants.   Most of the tentacles are wilted × 357.

Fig. 5.   Exconjugant devoid of tentacles.   The macronucleus is fragmented. × 357.

Fig 6.   Fusion of larva with adult.   Probably a case of precocious conju gation.   × 357.

Fig 7.   *Tokophrya lemnarum* devouring *Euplotes patella.*
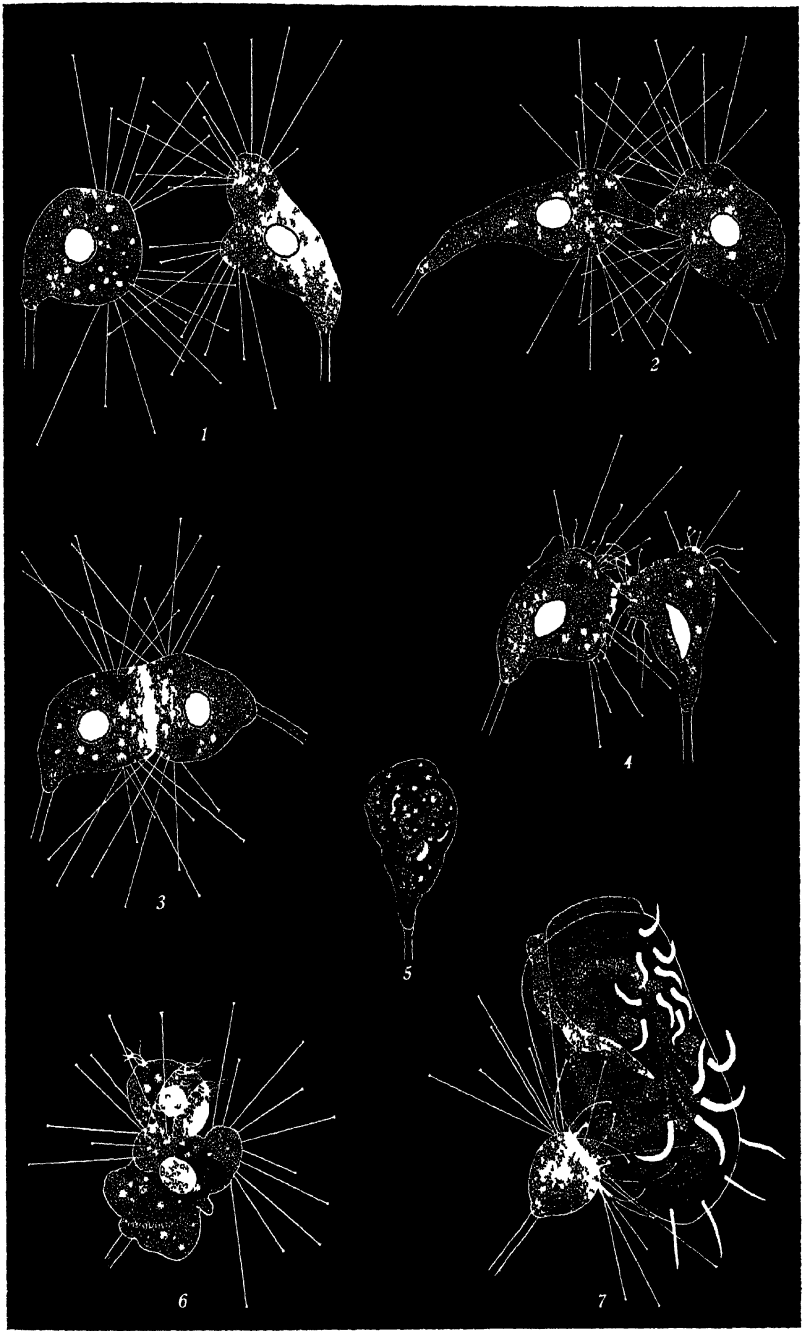
PLATE 35

Figs 1–3 Successive stages in the close of blood chamber after discharge of embryo ✕ about 1100

Fig 4 Tentacles of *Tolophrya lemnarum* (1) in the act of feeding, (2) fully extended, and (3) contracted ✕ about 4000

Figs 5, 6 Animals with flexures at different levels of their bodies ✕ 357

Figs 7–10 Successive stages of encystment ✕ 357
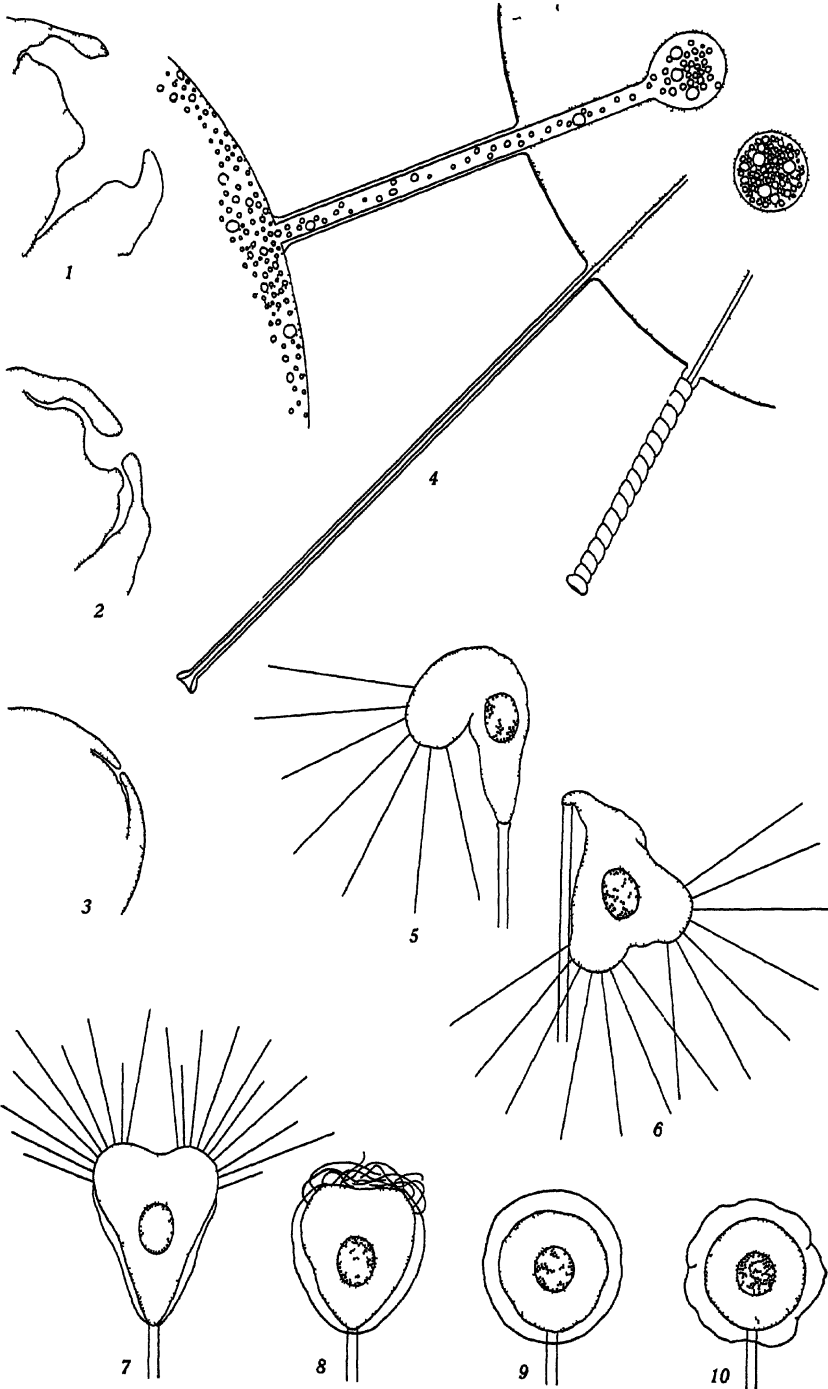
PLATE 36

Fig. 1. Pedicel of *Tokophrya lemnarum* showing (1) outer pellicular layer, (2) inner sheath of rods (striations), (3) central core, (4) basal disc, and (5) funneled region of attachment to body. S. H. × 3700.

Fig. 2. *Tokophrya lemnarum* containing resting micronucleus adherent to macronuclear membrane. B. P. × 750.

Fig. 3. Eaily bud, showing budding of paiental macronucleus and two active micronuclei. S. F. × 750.

Fig. 4. Later budding stage showing dumb-bell shape of macronucleus. S. F. × 750.

Fig. 5. Completed bud lying within biood chamber. Micronuclei of both parent and bud are visible. B. P. × 750.

Fig. 6. Completed bud showing basal granules of cilia. The birth pore is shown communicating with the brood chamber. Fl. H. × 750.

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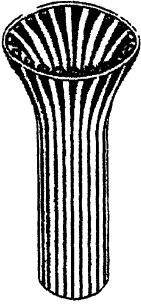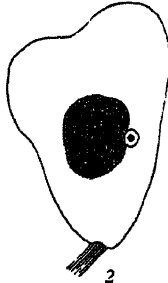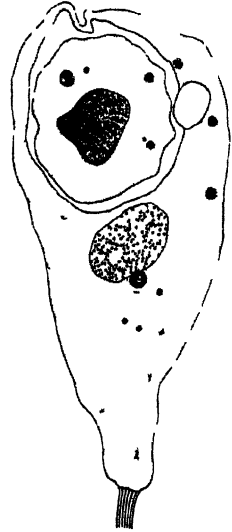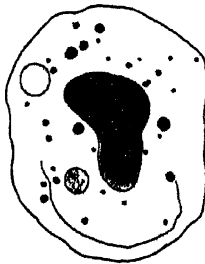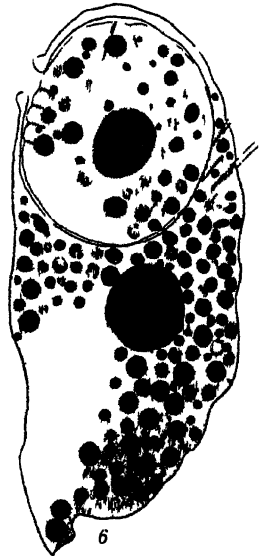
6

PLATE 37

Fig. 1. Early stage of conjugation. The micronucleus of each conjugant shows an incipient swelling. B. P. × 750.

Fig. 2. Conjugants showing (1) swelling micronuclei and (2) first prezygotic division. Fl. Saf. × 750.

Fig. 3. A pair of conjugants; one (right) showing products of two prezygotic divisions and a division of one of these products into two germ nuclei; the other (left) showing a passage of a germ nucleus through the separating membrane. S. F. × 750.

Fig. 4. Exconjugant showing first postzygotic division and minute particles (chromosomes?) within the micronuclei. S. H. × 750.

Fig. 5. Second postzygotic division in exconjugant. The macronucleus has elongated. S. F. × 750.

Fig. 6. Exconjugant showing the eight products of third postzygotic division. One of these products has undergone the final division to form permanent macronucleus and micronucleus. S. F. × 750.

Fig. 7. Exconjugant showing condensation of chromatin into the permanent micronucleus and early metamorphosis of macronucleus. The latter is recognized by three faint granules within it. S. F. × 750.

Fig. 8. Exconjugant with metamorphosing macronucleus and elongated old macronucleus in early stage of disintegration. Fl. H. × 750.

Fig. 9. Animal with normal macronucleus but still containing remnants of old macronucleus. S. F. × 750.
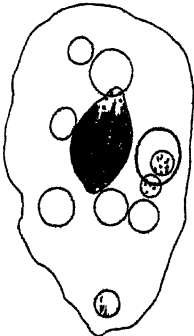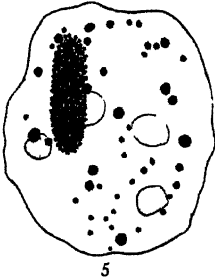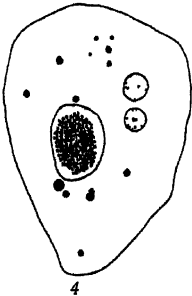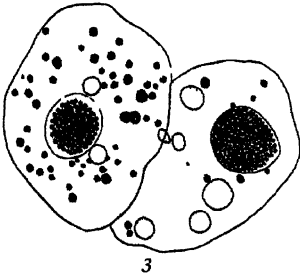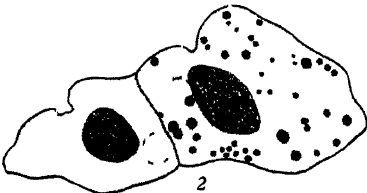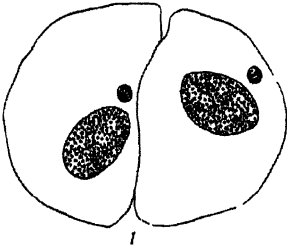
PLATE 38

Fig. 1. Animal showing old macronucleus reduced in size by absorption into cytoplasm. S. F. × 750.

Fig. 2. Macronucleus in early stage of metamorphosis. S. F. × 1500.

Fig. 3. Macronucleus in later stage of metamorphosis. S. F. × 1500.

Fig. 4. Typical functional macronucleus. S. F. × 1500.

Fig. 5. Macronucleus in early stage of budding to form nucleus of embryo. S. F. × 1500.

Fig. 6. Macronucleus in early stage of degeneration showing concentration of granules within its center. S. F. × 1500.

Fig. 7. Macronucleus with granules tending to arrange themselves in linear series, giving the striated appearance typical of a degenerating macronucleus. S. F. × 1500.

Fig. 8. Elongated macronucleus just prior to rupture of nuclear membrane. S. F. × 1500.

Fig. 9. Macronucleus with ruptured membrane causing egress of contained granules. S. H. × 1500.

Fig. 10. Remnants of old macronucleus after coalescence of escaped granules into vacuolated masses. S. F. × 1500.
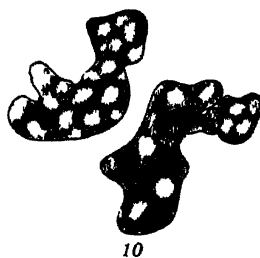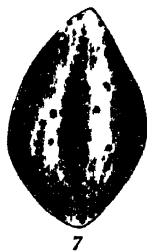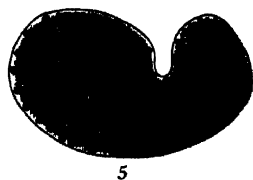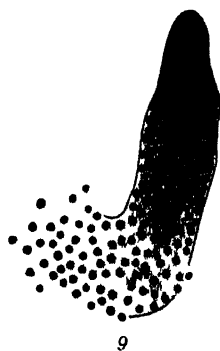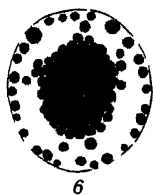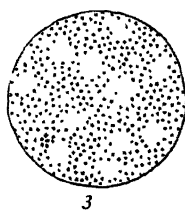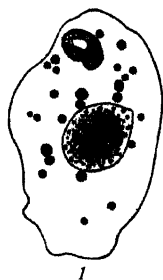
THE OCCURRENCE OF STREPTOSTYLY
IN THE AMBYSTOMIDAE

BY

THEODORE H. EATON, Jr

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In a study of some Pacific coast salamanders, it was found that at least three species of Ambystomidae possess a pivoting squamosal, which makes possible a slight forward and backward movement of the lower jaw and hyobranchial apparatus. Streptostyly, so far as the writer has been able to learn, is otherwise unknown in Amphibia. There is naturally no homology between the structural arrangements described here and those occurring in several groups of reptiles, since the bones are mostly different, and since the reptiles originated from Stegocephalian Amphibia, which lacked any such mechanism. Its presence, therefore, as an adaptive specialization in a few Urodela seems to warrant description.

This peculiarity was noticed in the following species: *Rhyacotriton olympicus* (Gaige), living in the mountain streams of the Olympic Peninsula and the Mount Rainier region of Washington; *Ambystoma gracile* (Baird) (synonym *A. paroticum* Baird), with a scattered distribution along the coast from northern California to southern British Columbia; and *A. macrodactylum* Baird, which ranges widely from northern California to British Columbia, Alberta, and Iowa. It is possible that one or two other species of *Ambystoma*, which were not available for dissection, such as *A. decorticatum* Cope, a rarity found along the coast from southeastern Alaska to Washington, may also be streptostylic, but the majority of the family, including *Dicamptodon*, have a fixed squamosal.

Material for study consisted of the following:

Rhyacotriton olympicus, 5 adults from the Olympic Peninsula, Washington
A. gracile, 4 adults from Washington, locality uncertain
A. macrodactylum, many adults, locality unknown
Dicamptodon ensatus, 3 adults, several larvae, from Marin County, California

Of this material two or three specimens of each species sufficed to give a satisfactory representation of the structures being studied, for there is little difference between individuals in the same stage of development.

The essential features of the original condition, from which this type of streptostyly was presumably derived, are illustrated in figure A, *1,*

which shows the right lateral aspect of the squamosal region of *Dicamptodon ensatus* Strauch. The prootic *(PO)*, the squamosal *(Sq)* and the quadrate *(Q)* together form a solid column to the base of which the mandible *(M)* articulates. From the anterior face of the squamosal projects the pterygoid *(Pt)*, running forward into a ligament which connects it with the maxillary. The posterior side of the squamosal is a
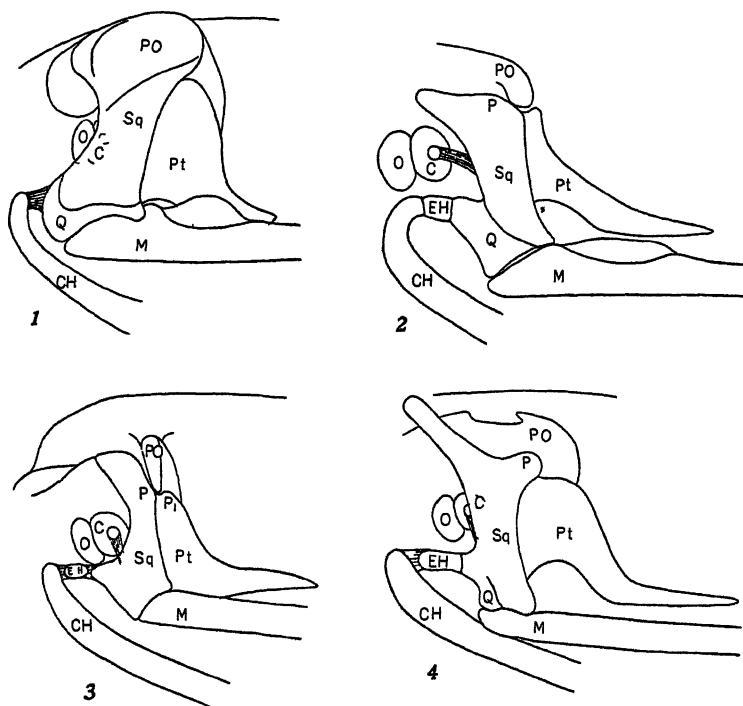
Figure A. Right lateral aspect of squamosal region of (1) *Dicamptodon ensatus* (Eschscholtz), (2) *Ambystoma macrodactylum* Baird, (3) *Ambystoma gracile* (Baird), (4) *Rhyacotriton olympicus* (Gaige).

C, columella; *CH*, ceratohyal; *EH*, epihyal; *M*, mandible; *O*, operculum; *P*, P_1, point where squamosal or pterygoid pivots on prootic; *PO*, prootic; *Pt*, pterygoid; *Q*, quadrate; *Sq*, squamosal.

crest, almost covering the two auditory bones, the operculum *(O)* and the columella *(C)*. The latter carries a stilus whose distal end is joined to the squamosal by a bit of connective tissue. Incidentally, the auditory apparatus is similar in every respect to that of most species of *Ambystoma*. This conclusion is based on the findings of Kingsbury and Reed (1909) and on dissections by the present writer. It does not confirm Dunn's statement (1922) that the two genera should be separated on

this basis, although other characters point to this separation. The cerato-hyal cartilage *(Ch)* is joined to the posterior heel of the quadrate by a ligament.

In the three streptostylic species, several modifications of this ar-rangement have taken place. The squamosal, pterygoid and quadrate pivot as a unit at either one or two points *(P, P_1)* on the prootic. These points are marked by a more or less distinct oval face on the prootic when the pivoting bones have been removed. In *A. gracile* and *A. macrodac-tylum* the prootic forms a small crest just over the squamosal, evidently serving as a fulcrum for its movement. The greater length of the liga-ment from the columella in these two species, compared with *Dicamp-todon,* allows the squamosal to move, since the columella itself is sta-tionary. Hence the length of this ligament serves as a measure of the distance that the squamosal can move either forward or backward, namely, one or two millimeters.

The quadrate, strangely enough, ceases to provide the whole articula-tion for the lower jaw, but comes to lie farther posteriorly, and the squamosal takes on a part of this function. In *A. gracile* no quadrate was visible. It has apparently fused with the squamosal. In *A. macrodac-tylum* it is large and distinct. The case in *Rhyacotriton* is questionable. A partial suture was found in this genus, but it may not appear in all specimens. Whereas *Dicamptodon* has no cartilage between the cerato-hyal and the quadrate, the other three have a small piece lying in or replacing the ligament, which is evidently the epihyal *(Eh),* (see Parker, 1882). Such a cartilage occurs in the larva of *Dicamptodon,* but it forms a horn of the hyoid arch which reaches considerably past (dor-sad to) the point of articulation, and so is lost when this horn atrophies at metamorphosis.

In the musculature of the squamosal region there is considerable dif-ference among the three genera. All, however, have a strong oblique pterygoideus muscle running from the lateral face of the pterygoid, just anterior to the squamosal, to insert on the inner face of the mandible. In *Dicamptodon* the masseter originates partly on the anterior face of the squamosal, but largely on the prootic crest above. In the two species of *Ambystoma* its origin is entirely on the squamosal, hence it acts with the pterygoideus to elevate the mandible and reduce the angle between the latter and the squamosal. In *Rhyacotriton* there is a long oblique process on the posterodorsal angle of the squamosal, and a part of the masseter originates on the anterior edge of this process, the remainder along the vertical anterior face of the squamosal. The effectiveness of the masseter

in pivoting the squamosal is thus increased by the additional leverage. The only other muscle entering this complex is the digastric, which originates on the superficial fascia of the dorsal bundle of longitudinal body muscles behind the head, and tapers to an insertion on the base of each mandible just below the articulation of the latter with the skull. Its action is, as usual, to depress the mandible and open the mouth. Whereas a few fibers at its origin come from the otic capsule in *Dicamptodon* and *Ambystoma,* with the great majority on the fascia described above, in *Rhyacotriton* fully two-thirds of the origin of the digastric is on the posterior margin of the horn of the squamosal. Therefore the digastric opposes the masseter both at its origin and at its insertion, and in action would serve not only to depress the mandible but to pull back the dorsal end of the squamosal, pushing forward its ventral end with the mandible and hyoid cartilage attached.

The mechanism for moving the squamosal, pterygoid, and quadrate, and with them the visceral arches, is therefore better developed in *Rhyacotriton* than in the others. The three species possessing this form of streptostyly do not, however, fit in a linear series. *A. macrodactylum* is most like other species of *Ambystoma* in showing a large, distinct quadrate, and the motion of the apparatus seemed to be less free in a dissected specimen than in *A. gracile* or *Rhyacotriton*; but, in common with *A. gracile,* it possesses the prootic flange or fulcrum in front of the pivot, which *Rhyacotriton* and the non-streptostylic species lack. Again, *A. gracile* apparently has lost a separate quadrate by fusion with the squamosal, while the others retain it. In many other morphological characters *Rhyacotriton* is far removed from the rest of the Ambystomidae, but its retention of so unique an adaptation as a movable squamosal, in common with two species of *Ambystoma* which have the same general distribution, suggests that it evolved from an isolated stock of true *Ambystoma,* and that its more primitive characters, such as the presence of a lacrimal, are due to a relative retardation of development.

A comparison of the streptostylic condition in these salamanders with that in certain reptiles, for example, Lacertilia (Bradley, 1903), shows great differences in both skeleton and muscles, such that no homology is conceivable. In the lizards there is a slight movement possible between the occipital and frontal portions of the skull, so that the upper jaw can be elevated. The quadrate is long, columnar, articulated at its upper end with the posterior edge of the frontal section of the skull, and at its lower end with both the pterygoid and the mandible. Each of these articulations is a movable joint. When the mouth is opened a pair of deep

short muscles, the pterygo-sphenoidales posterior, running outward and downward from the basisphenoid (occipital segment) to the rear end of the pterygoids, contract and pull the pterygoids inward and forward. These in turn push up the frontal segment of the skull, and draw the mandibles and lower ends of the quadrates forward with them. Most lizards are provided with pterygo-parietales muscles which, as the mouth is closed again, abduct the pterygoids from their previous position, allowing the upper jaw to fall back to its usual level. Other kinds lack these muscles, and it is assumed that they are less essential than the pterygo-sphenoidales posterior, since the frontal segment in such cases may drop by its own weight. The quadrates play a passive part in this mechanism, simply supporting the posterior end of the mandibles and pterygoids. Other movements, such as rotation of the two mandibles upon their own axes, are shown to be possible, but further discussion here is unnecessary.

In applying the name streptostyly to the condition in the salamanders, this term cannot be defined as the possession of a movable quadrate, although that would be sufficient among reptiles, and has even been used for mammals, on the ground that the incus, representing the quadrate, is no longer fixed in the cranium. But it is perhaps more satisfactory (in lieu of inventing a new word) to define streptostyly as the movement of the lower jaw upon the cranium by means of a movable intermediary bone, and to consider that the loose incus of mammals may be a product of a past occurrence of streptostyly.

This apparatus has not been observed to function in salamanders because it has only been studied in the preserved specimens; but we may at least infer that it helps to force large prey into the mouth. Contraction of the masseter and pterygoideus elevates the mandible, pressing the teeth into or against the prey. This resistance would automatically swing the lower end of the squamosal back and draw the mandible and the prey in the same direction, because the action of the above-mentioned muscles reduces the angle between mandible and squamosal. The upper end of the squamosal is held at the pivoting point, which allows only the lower end to move, and permits backward motion only. The action of the digastric is antagonistic to these, as previously shown, especially when it originates on the squamosal itself above the pivot, as in *Rhyacotriton*. This action would push the lower end of the squamosal, bearing the mandible, forward again, at the same time releasing the teeth from the body of the prey (on account of depression of the mandible), so that a new grip could be taken farther out. In this way any large and active prey would be forced, little by little, into the mouth of its captor.

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